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An investigation into the role of bone mass and other factors in determining fracture risk in children

AN INVESTIGATION INTO THE ROLE OF BONE MASS AND OTHER FACTORS IN DETERMINING FRACTURE RISK IN CHILDREN

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ABSTRACT

Background

Fractures in children are common and increasing in incidence. Previous research into the effects of bone mass and other determinants of fracture risk in children has been mainly of a cross-sectional or case-control study design, and there is a need for detailed prospective data to explore the specific roles of volumetric bone density and bone size.

Aims

- To carry out a systematic review and meta-analysis of the previously published evidence for an association between bone mass and fractures in children
- To investigate the confounding structure of childhood bone mass using a contemporary geographical birth cohort
- To prospectively investigate volumetric bone density and bone size as determinants of childhood fractures
- To investigate other determinants of childhood fracture risk that may act independently of bone mass.

Methods

A dual energy X-ray absorptiometer (DXA) scan was performed in 5933 children at aged 9.8 years from the Avon Longitudinal Study of Parents and Children. Reported fractures were collected over the next 24 months. Data had been previously collected on variables such as gender and socio-economic status. Variables were analysed to see if they affected fracture risk by an action that was independent of bone mass. A novel method of using humerus data from total body DXA scans was developed to allow exploration of biomechanical strength. Data were analysed using t-tests and multivariable regression.

Results

Of the 5933 children, 527 (8.9%) reported at least one fracture over the two-year follow-up period. Per standard deviation (SD) decrease in estimated volumetric density, fracture risk in children approximately doubled over the following two years (OR 1.96, 95%CI 1.27 to 3.01, P=0.002). Per SD decrease in bone size relative to body size, fracture risk increased by 62% (OR 1.62, 95%CI 1.23 to 2.15). Family size, gender, ethnicity, physical activity and birth-weight were risk factors for fractures that were independent of bone mass.

Conclusions

Bone fragility as measured by volumetric bone density and bone size relative to body size, are determinants of fracture risk in children. However, other risk factors acting via increased exposure to injuries or via the mechanism of injury are also important.

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AUTHOR'S DECLARATION

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original, except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other academic award. Any views expressed in the dissertation are those of the author.

SIGNED: Emma Clark ..... DATE: 23d March 2007

LIST OF ABBREVIATIONS

Abbreviation	In Full
ADHD	Attention deficit hyperactivity disorder
A&E	Accident and Emergency
AGA	Appropriate for gestational age
AGES	Age, Gene/Environment Susceptibility study
ALP	Alkaline phosphatase (also known as BAP)
ALSPAC	Avon Longitudinal Study of Parents and Children
ANOVA	Analysis of variance
AR	Aspect ratio
AVU	Apparent velocity of ultrasound
BA	Bone area
BAP	Bone alkaline phosphatase (also known as ALP)
BBRI	Bending breaking resistance index
BGP	Bone Gla protein (also known as OC)
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit of bone
BTT	Bone transmission time
CI	Confidence interval
CiF	Children in Focus
COL1A1	Collagen type I gene coding for first alpha peptide
COL1A2	Collagen type I gene coding for second alpha peptide
COREC	Central Office for Research Ethics Committee
CSA	Cross sectional area
CSMI	Cross sectional moment of inertia
CT	Computed tomography
CXR	Chest X-ray
DAWBA	Developmental and Well-Being Assessment
DEQCT	Dual energy quantitative computed tomography
DNA	Deoxyribonucleic acid
DPD	Deoxypyridinoline
DSM	Diagnostic and Statistical Manual
DTI	Department of Trade and Industry
DXA	Dual energy X-ray absorptiometry
ERα	oEstrogen receptor alpha
F	Female
FFQ	Food frequency questionnaire

Abbreviation	In Full
GH	Growth hormone
GP	General practice
GPRD	General practice Research Database
HASS	Home Accident Surveillance System
IBD	Inflammatory bowel disease
ICD	International Classification of Diseases
IGF	Insulin-like growth factor
IGF-BP	Insulin-like growth factor binding protein
IGF-R	Insulin-like growth factor receptor
IQR	Inter-quartile range
IUGR	Intra-uterine growth retardation
JCA	Juvenile chronic arthritis
JIA	Juvenile idiopathic arthritis
L	Lumbar
LASS	Leisure Accident Surveillance System
LRP5	Low density lipoprotein receptor-related protein 5
LRT	Likelihood ratio test
M	Male
MHRA	Medicines and Healthcare Products Regulatory Agency
mg	miligram
MRC	Medical Research Council
MRI	Magnetic resonance imaging
mSV	mili-Sieverts
N	North
NCP	Non-collagenous proteins
NHS	National Health Service
NMS	National Morbidity Statistics
OC	Osteocalcin (also known as BGP)
OPCS	Office of Population Censuses and Surveys
OR	Odds ratio
PICP	a Type I collagen extension propeptide
PINP	a Type I collagen extension propeptide
PPARγ	Peroxisomal proliferator-activated receptor gamma
pQCT	Peripheral quantitative computed tomography
PTH	Parathyroid hormone
PYD	Pyridinoline

Abbreviation	In Full
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RCT	Randomised controlled trial
RR	Relative risk
RTI	Road traffic injury
S	South
S-CTX	Serum C-terminal cross-linking telopeptide of type I collagen
SD	Standard deviation
SE	Standard error
SEQCT	Single energy quantitative computed tomography
SGA	Small for gestational age
SOS	Speed of sound
SMD	Standardised mean difference
SNP	Single nucleotide polymorphism
S-NTX	Serum N-terminal cross-linking telopeptide of type I collagen
TB	Total body
TBLH	Total body less head
TGF-β	Transforming growth factor-beta
TRACP	Tartrate resistant acid phosphatase
TRACP-5b	5b-isomer of tartrate resistant acid phosphatase
TV	Television
UBHT	United Bristol Healthcare Trust
U-CTX	Urinary C-terminal cross-linking telopeptide of type I collagen
UK	United Kingdom
U-NTX	Urinary N-terminal cross-linking telopeptide of type I collagen
US	United States
USA	United States of America
VDR	Vitamin D receptor
WHO	World Health Organisation
Z	Section modulus

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CHAPTER 1: PROJECT RATIONALE, OVERVIEW AND OBJECTIVES

1.1. PROJECT RATIONALE

Fractures in children are common. The reported incidence in the UK ranges from 1.6% (5) to 3.6% (6) per year, and the lifetime risk of sustaining a fracture in childhood for boys is 42-64% and for girls is 27-40% (4,7). The incidence appears to be increasing over time (8). There is a peak in fracture risk around the age of 14 years in boys and 11 years in girls (2). Although previous investigations into the public health impact of fractures have largely focussed on elderly populations, the incidence of fractures during childhood is similar to that in the elderly. For example the incidence of fractures of the ulna and radius in boys aged 5 to 15 years in the UK in 1991-1992 was 23 per 10,000 compared to 24 per 10,000 in men aged 85 and over (9). Fractures in children can affect health and development as a consequence of complications such as mal-alignment of the fractured bone, limb overgrowth (10) and acute compartment syndrome (11). Childhood fractures also result in time off school, activity restricted days (14 and 26 days for arm and leg fractures respectively) (12) and can have long-term consequences arising from complications such as secondary osteoarthritis (13).

Fractures in children are generally thought to reflect the fact that falls and other injuries are common in childhood (14), but there is emerging evidence from case control studies that fractures in childhood are related to underlying skeletal fragility (15-24). Measuring bone fragility in vivo is difficult and in general two surrogate components are used: volumetric bone density and bone size. Determinants of bone fragility and fracture risk in childhood will be a complex interaction of genetic and environmental factors on both volumetric bone density and bone size. Although some work has been carried out in this area it is mainly of a cross-sectional or case control study design, and there is a need for detailed prospective data to examine the determinants of fracture risk in children and to explore the specific roles of bone density and bone size.

1.2. AIMS

The overall aim of this project is to understand the aetiology of fractures in childhood, and in so doing to inform population-based interventions aimed at reversing the recent increase in incidence. The specific aims are to

1. Carry out a systematic review and meta-analysis of the current evidence for an association between bone mass and fractures in children.
2. Investigate the confounding structure of childhood bone mass using a contemporary geographical birth cohort.
3. Prospectively investigate bone size and bone density as determinants of fractures in childhood using a contemporary geographical birth cohort.
4. Investigate other determinants of fracture risk in childhood that may act independently of bone mass using a contemporary geographical birth cohort.

1.3. PROJECT OVERVIEW

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a geographical birth cohort of over 14 000 children. Data have been collected prospectively from questionnaires, medical records and from direct measurement of the children. Parental data have also been collected. For this project, bone mass was measured by dual energy X-ray absorptiometry (DXA) on the cohort at aged 9.8 years. Prospective data were then collected on reported fractures over the following two years by questionnaires. Fractures were verified by examination of X-ray reports where available.

The confounding structure of bone mass was investigated, with a focus on the social determinants of bone mass and fat mass as a determinant of bone mass, as little or no data has been previously published on these areas. A novel method was developed to allow direct measurement of the humerus size and shape from total body DXA scans, from which biomechanical strength indices were estimated. The early life, child (excluding bone mass), maternal and paternal determinants of fractures in childhood were investigated. Finally, the associations between bone density, bone size and biomechanical strength indices at aged 9.8 years and risk of fracture over the following two years were examined.

1.4. PROJECT OUTLINE

This thesis is divided into four sections: a literature review, a methods sections, results and discussion, and contains the following:

- Literature review of the structure, growth and material properties of bone, and the definition of, and techniques available for measuring bone mass and osteoporosis (*Literature Review, Chapter 2*)

- Literature review of the determinants of bone mass in childhood (*Literature Review, Chapter 3*), of childhood injury and fracture epidemiology (*Literature Review, Chapter 4*) and of the determinants of fractures in children (*Literature Review, Chapter 5*)

- Systematic review and meta-analysis of the association between bone mass and fracture risk in children (*Literature Review, Chapter 6*) with a summary (*Literature Review, Chapter 7*)

- Methods of ALSPAC, specific methods of this fracture study including development of the novel method for measuring humerus size and shape from total body DXA scans, and general statistical methods (*Methods, Chapters 8 to 10*)

- Description of the variables used in this project *(Results, Chapter 11)* and a description of the confounding structure of estimated bone density and bone size measured at aged 9.8 years (*Results, Chapter 12*).

- An investigation into the early life, child (excluding bone mass), maternal and paternal determinants of childhood fractures at aged 9.8 years (*Results Chapter 13*).

- An investigation into the association between estimated volumetric total body bone density, bone size and biomechanical strength indices measured at aged 9.8 years and fracture risk over the following two years (*Results, Chapter 14*).

- Discussion of the results, update of the meta-analysis, weaknesses of this study, policy implications and further work needed *(Discussion, chapter 15)*.

CHAPTER 2: BONES

This chapter is divided into four sections. The first reviews the structure of bone; the second the growth of bones; and the third the material properties of bone. The last section focuses on the definition of bone mass and techniques for measuring this.

2.1. STRUCTURE OF BONE

Bone is a composite material consisting of protein (collagen and Non-Collagenous Proteins, NCPs), mineral, water, polysaccharides, living cells and blood vessels (25). There is a structural hierarchy of bone: molecular; micro-architectural; and macro-architectural. At the molecular level is the collagen fibril with its associated mineral. Above this, the micro-architecture of bone exists in two fairly distinct forms: woven bone and lamellar bone. At the macro-architecture level bone is generally organised in two different ways: cortical (compact) bone and trabecular (cancellous) bone. The gross structure of a human long bone consists of the shaft or diaphysis, the ends or epiphysis, and in children the metaphysis and growing-plate. See Figure 1 on page 22.

2.1.1. The cells of bone

There are various cells within bone: bone-lining cells; osteoblasts; osteocytes; and osteoclasts.

2.1.1.1. Bone-lining cells

These cover all surfaces on bone including the blood vessel channels. The layer of cells on the outside of bone is called the periosteum, and the layer on the inside of bone is called the endosteum. These cells are derived from the stem cells that generate all bone cells called the osteoprogenitor cells.

Figure 1: Gross structure of a long bone

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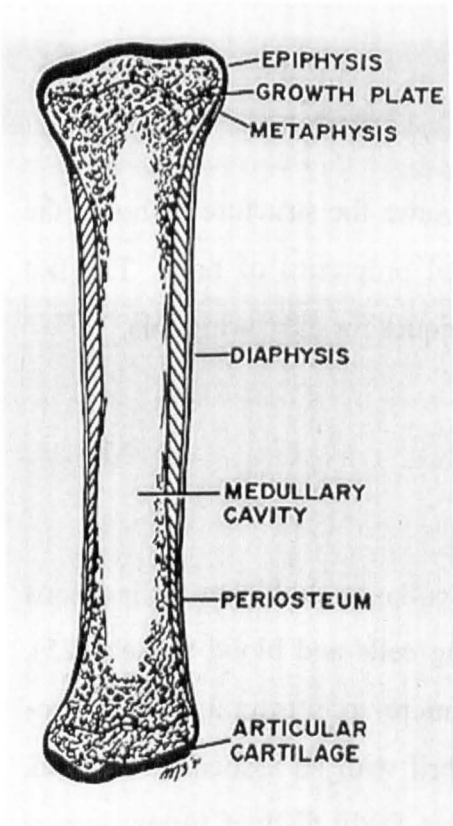


2.1.1.2. Osteoblasts

Osteoblasts derive from bone-lining cells and are responsible for the formation of bone. They initially lay down the osteoid (a matrix of collagen plus a few other organic compounds), in which mineral is later deposited. Osteoblasts probably also have a role in mineralisation.

2.1.1.3. Osteocytes

Osteocytes derive from osteoblasts. They are imprisoned within the hard bone tissue and connect with neighbouring osteocytes and with bone-lining cells by processes that are housed in little channels called cannaliculi.

2.1.1.4. Osteoclasts

Osteoclasts are bone destroying cells. They are large multinucleated cells derived from precursor cells circulating in the blood. They clamp themselves to the bones surface and leave a space underneath a ruffled border. Beneath this border the bone is dissolved, and

debris is packed into vesicles that pass through the cell body of the osteoclast and are emptied into the space above. When osteoclasts have done their job they disappear and presumably die.

2.1.2. Bone at the molecular level

2.1.2.1. Collagen

There are four types of collagen within the human body (Type I to IV). The collagen of bone is Type I. The protein molecule tropocollagen is formed on ribosomes within cells, and is connected by means of disulphide cysteine links. Tropocollagen consists of three polypeptides of the same length – two alpha1-polypeptides and one alpha2-polypeptide. These polypeptides contain great stretched repeats of Glycine-X-Y, with X often being proline and Y sometimes hydroxyproline. Once tropocollagen leaves the cell the ends of the joined polypeptides are snipped off (which contains the disulphide bonds), resulting in the three chains being held together by hydrogen bonds in a characteristic left-handed triple helix. The result is an inflexible polypeptide 300nm long (26).

The tropocollagen molecules line up and form bonds with molecules in neighbouring files to form microfibrils. The tropocollagen molecules alongside each other are staggered by about a quarter of their length. There is a gap between the head of one molecule and the tail of the next – the hole region. The whole microfibril becomes stabilised by intermolecular cross-links. These cross-links are of two types: ones which are reducible with sodium borohydride; and ones which are non-reducible (27). Microfibrils aggregate to form fibrils, although the exact arrangement is unknown (28).

2.1.2.2. Non-Collagenous Proteins (NCPs):

NCPs comprise about 10 to 15% of the protein in bone. Some of these proteins almost certainly have a role in the initiation and control of mineralisation or reconstruction, and some may have a role in binding the collagen and mineral together. Osteocalcin, or Bone Gla Protein (BGP) is found exclusively in bone tissue and makes up approximately 10-20% of the NCPs. While the *in vivo* function of osteocalcin is not known, its level in blood reflects bone turnover, and is used for monitoring bone turnover in clinical settings (29).

2.1.2.3. Bone mineral

Some of the mineral in bone is a version of calcium phosphate called hydroxyapatite. However, the crystals are impure and around 4-6% have carbonate replacing the phosphate groups, making the mineral more truly a carbonate apatite (dahlite). This carbonate substitution takes place near the edges of the bone, close to the vascular and marrow spaces, and tends to reduce the crystalinity of the crystals (30). The crystalline mineral in bone is more likely to be plate-shaped (arranged as platelets) than needle-shaped (25).

Bone mineralises on surfaces. Initially a matrix of collagen plus a few other organic compounds is laid down (called the osteoid). Mineral is then deposited in parts of the fibrils that are high in hydrophilic residues, possibly the hole region (31). In this early part of the mineralisation process there appears to be a very uniform structure. Later the mineral is deposited all over the collagen fibrils, and also within them. The plate-like mineral crystals forms quite large lumps as they grow, but the individual mineral crystals tend to be oriented in the direction of the collagen fibrils. Later mineral is deposited between the fibrils.

2.1.3. Bone at the micro-architectural level

2.1.3.1. Woven bone

Woven bone is characteristically present in the foetus and in callus formation at the site of fracture repair. It is laid down very quickly and the collagen fibrils are arranged randomly (32). Woven bone is highly mineralised, but is often porous at the micron level because of mineral free spaces and extensive spaces surrounding the osteocytes and blood vessels.

2.1.3.2. Lamellar bone

Lamellar bone is laid down more precisely and slowly than woven bone (33). The collagen fibrils and their associated mineral are laid down in sheets (lamellae), and the

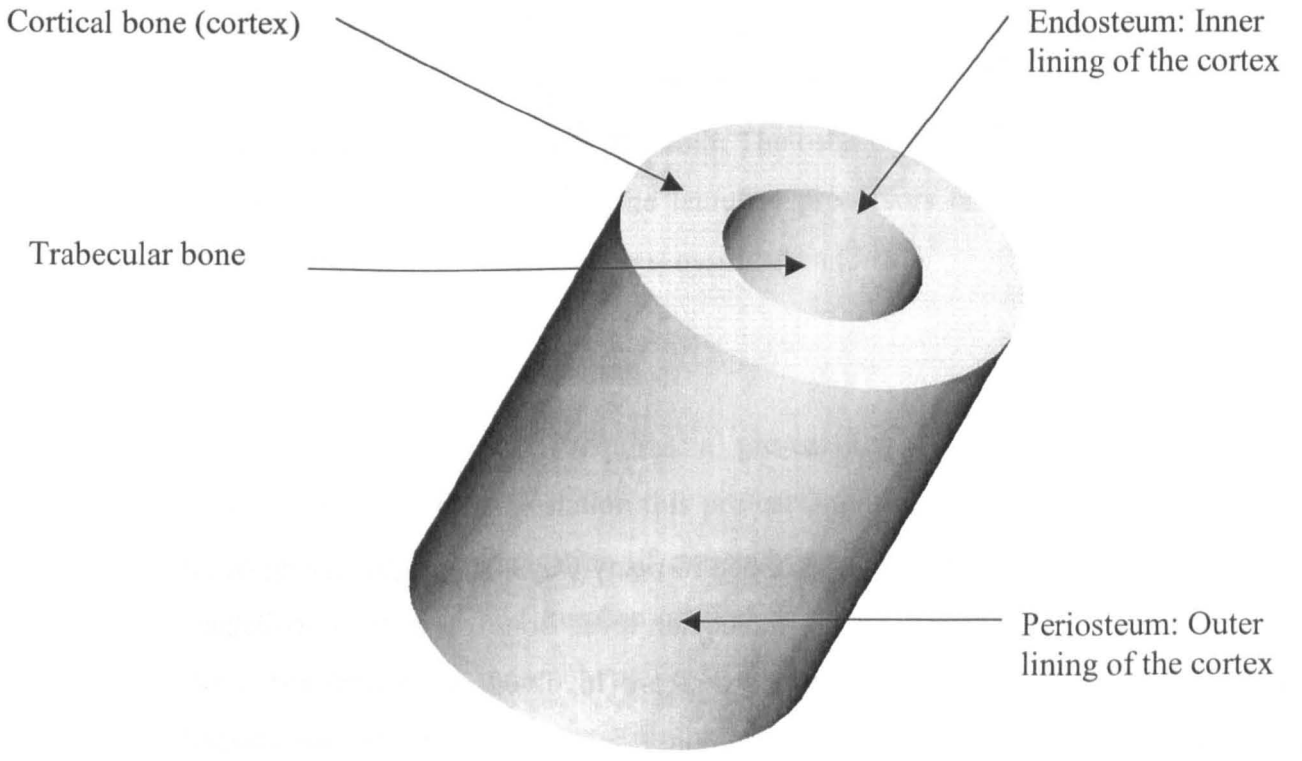
mineral appears to be orientated in the plane of the circumferential extension of the lamellae. Lamellar bone is less mineralised than woven bone.

2.1.4. Bone at the macro-architectural level

2.1.4.1. Cortical bone

Cortical bone is solid, with the only spaces in it being for osteocytes, blood vessels or erosion cavities. It is generally found in a thin layer on the outside of bones (see Figure 2, below).

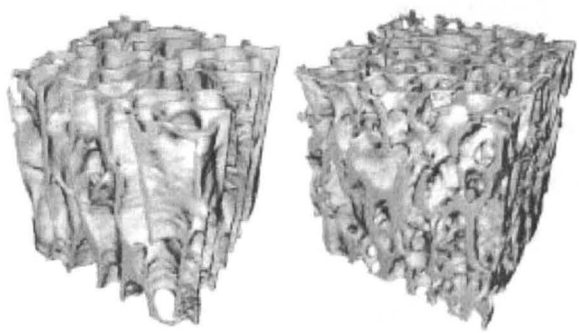
Figure 2: Cross-section of a long bone.



2.1.4.2. Trabecular bone

Trabecular bone contains large spaces and is arranged into struts, or trabeculae. See Figure 3, below. The spaces between the trabeculae are usually filled with marrow where blood cells such as erythrocytes (red cells) leucocytes (white cells) and platelets are formed. Trabecular bone is also known as spongy bone or cancellous bone.

Figure 3: Scanning electron micrograph of normal trabecular bone. Reprinted with kind permission from Prof Tony Keaveny, Berkeley University. Image available at http://www.npaci.edu/envision/v15.3/keaveny.html



2.1.5. Summary

Bone is a complex material that can be described in many ways. It contains living cells as well as mineral and proteins. At the molecular level bone consists of collagen arranged in fibrils; non-collagenous proteins that are involved in mineralisation or reconstruction; and bone mineral, most commonly hydroxyapatite. At the micro-architectural level bone may be in a woven pattern or a more lamellar-type pattern. At the macro-architectural level bone may be divided into cortical bone found in a thin layer on the outside of long bones; or trabecular, spongy bone found on the inside. The next section reviews the growth of bones during embryology and childhood.

2.2. GROWTH OF BONES

This section focuses on bone growth at the cellular and macroscopic level, and then discusses change in bone size and shape during three stages: fetal life, childhood and adult life.

2.2.1. Bone growth at cellular level

Bones develops in two ways: intramembranous ossification (the skull and facial bones) and endochondral ossification (the remainder of the skeleton). This mainly occurs during fetal life, but also to a lesser extend during childhood.

2.2.1.1. Intramembranous ossification

Intramembranous ossification begins with a layer of mesenchymal cells. This membrane becomes highly vascular and the mesenchymal cells differentiate into isolated osteoblasts that begin to secrete osteoid. The osteoid matrix is mineralised at the end of the embryonic period to form the lamellae precursors or bony spicules. There is no cartilage model in intramembranous ossification (34).

2.2.1.2. Endochondral ossification

Endochondral ossification requires a pre-existing cartilaginous model called the analgen. By five weeks gestation this pre-cartilaginous anlagen has been laid down by the migration and condensation of mesenchymal cells (35). These anlagen reflect the shape, size, position and number of skeletal elements, which will be present in the mature skeleton. The mesenchymal cells then differentiate into chondrocytes and form the cartilage model on the existing anlagen template. This cartilaginous model then undergoes invasion by osteoblasts and is subsequently mineralised.

2.2.2. Bone growth at the macroscopic level

2.2.2.1. Modelling

In modelling the gross shape of the bone may be altered. This occurs by adding or taking away bone at the periosteal or endosteal surfaces. Long bones can grow width-ways (appositional growth) (36) and length-ways (longitudinal growth).

2.2.2.2. Remodelling

In remodelling, the area of bone affected is usually a small individual packet called a basic multicellular unit (BMU) and can occur anywhere including the internal body of the bone, by the formation of Haversian systems or secondary osteons (37). The Haversian systems are approximately 200 μm in diameter (25). Haversian systems probably occur when calcium needs to be released into the blood stream for the purpose of mineral homeostasis (38), or they may play a role in repairing microdamage (39).

2.2.3. Bone growth during fetal life

During the embryonic stage of life the foetal skeleton is formed by intramembranous ossification and endochondrial ossification. (See page 27).

2.2.4. Bone growth during childhood and adolescence

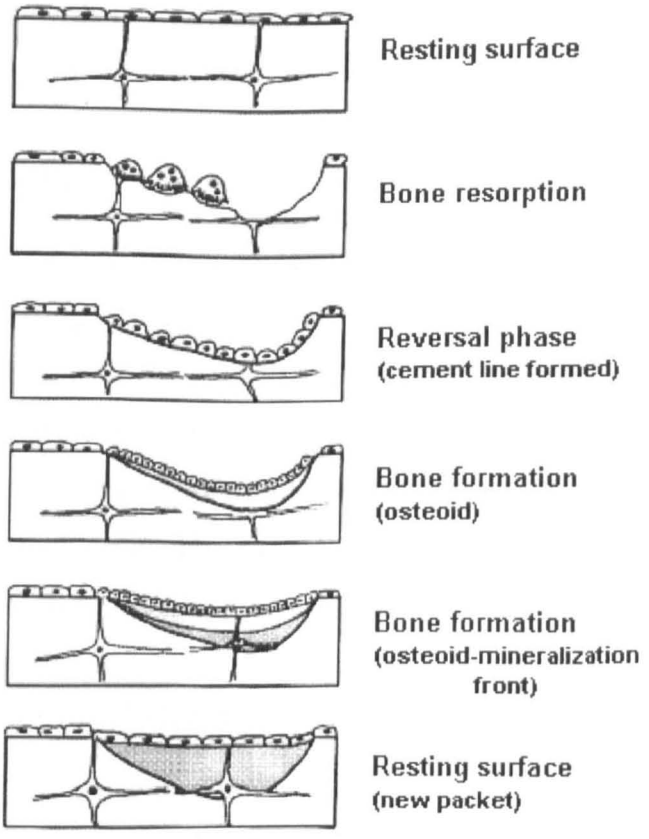
Bones grow during childhood and adolescence mainly by modelling (see above), but some bones are still being formed by intramembranous and endochondrial ossification (see page 27).

2.2.5. Bone remodelling during adult life

During adult life bones are constantly remodelled by the formation of Haversian systems (see above). These occur in the cortex of bones where giant Haversian systems may form due to the coalescence of many small Haversian systems. At the hip these giant systems have been linked to an increased risk of fracture (40) due to increased cortical porosity (see page 32).

Remodelling also occurs on the trabeculae of bone (41). Here there is a constant cycle (the remodelling cycle) (42) of bone resorption on the surface layer by osteoclasts followed by the laying down of a cement layer and then synthesis of unmineralised osteoid by osteoblasts to fill the resorption cavity. The osteoid is then mineralised, the bone surface finally being covered by lining cells and a thin layer of unmineralised osteoid. See Figure 4 below. This balance between bone resorption and bone formation is normally maintained by factors secreted by osteoblasts such as matrix metalloproteinases and factors released from bone by osteoclasts such as transforming growth factor beta (TGF-β) (43). This balance is disrupted in osteoporosis, where bone resorption out-weights bone formation and results in an increased risk of fracture (see page 41).

Figure 4: The bone remodelling cycle. Adapted from a figure available at http://www.lab.anhb.uwa.edu.au/mb140/MoreAbout/bonedynamics.html



2.2.6. Summary

Bones grow in different ways depending on whether the skeleton is developing in-utero, during childhood or in an adult who has reached their final height. In a foetus the skeleton develops in two ways: intramembranous ossification for the skull and facial bones; and endochondral ossification for the remainder of the skeleton. In growing children, bones elongate and change shape by modelling, where bone is added or taken away from the periosteal or endosteal surfaces. In adults, bones are constantly remodelled by the formation of Haversian systems in the cortex, and the remodelling cycle in trabeculae.

2.3. MATERIAL AND GEOMETRIC PROPERTIES OF BONES AND THEIR RELATION TO FRACTURES

Bone has to be able to withstand both tensile and compressive forces and so the main mechanical properties bone needs are elasticity and stiffness without being brittle. The ability of bones to resist fracture depends on their mass, material properties, micro-architectural arrangement, geometry and mechanical properties (44). Bone mass will be covered in the next section (Section 2.4, page 41). This section covers material properties of bones, macro-architecture, mechanical properties and overall bone geometry.

2.3.1. Material properties of bone

The main constituents of bone that contribute to fracture prevention are the crystal (mineral) component and collagen. As there is such an intimate relationship between the two at the nanometer level, it is somewhat artificial to consider each separately, but doing this can provide clues to important properties of bone.

2.3.1.1. Bone mineral

The majority of mineral in bone is hydroxyapatite (see page 24). With increasing age changes occur in bone mineralisation such that the mineral platelets coalesce and become deposited all over the collagen fibrils and also within them and within the extra-cellular matrix (25). It has been shown that mineral platelet size, particularly the aspect ratio (length to width), is directly related to bone toughness i.e. the ease with which a crack propagates (45), and there is a general tendency for tensile strength (see page 35) to increase with increasing mineralisation (25).

2.3.1.2. Collagen arrangement

The orientation of collagen fibres (see page 24) is an important determinant of the mechanical behaviour of bone. Studies on the radial cortex of horses have shown that orientation of the fibres with respect to the long axis of bone, and with respect to normal forces that will occur during walking are strongly associated with fracture resistance

(46,47). Also, the cross-linkages between the collagen microfibrils appear to be important in fracture prevention. These cross-links are of two types (see page 23) and the ratio of these appears to contribute to bone fragility: one study reported a different ratio in the reducible to non-reducible collagen cross-links in postmenopausal women with osteoporosis compared to normal controls (48).

2.3.2. Macro-architectural arrangement of bone

This section discusses the role of cortical architecture (thickness and porosity) and trabecular architecture (connectivity) in fracture resistance of bone.

2.3.2.1. Cortical architecture

Cortical thickness

Cortical bone is less porous than trabecular bone, and therefore contributes to reducing fracture risk. Thinner cortical widths found using metacarpal morphometry were associated with wrist and forearm fractures in one study of children (22). In this study, the cortical width of metacarpal bones of 321 children aged 9 to 16 years with upper limb fractures was on average smaller than that of the 321 controls matched for gender (cortical width of 3.3mm ± 0.7 in fractures compared with 3.5mm ± 0.8 for controls, $P<0.001$ for difference after adjustment for age and weight differences).

Thinner cortices of the femur have also been shown to be associated with an increased risk of stress fracture in a study of military recruits (49). In this study on 693 female US Marine Corps recruits, those who experienced stress fractures during training had an approximately 5.7% smaller cortical width at the femur compared to controls ($P=0.0033$).

Cortical porosity

The cortex of bones is not a solid sheet but has pores (see page 25) within it for osteocytes, blood vessels or erosion cavities. The number of pores within the cortex can increase as a normal part of growth, perhaps to supply the increased calcium needs during adolescence (50), due to the formation of giant Haversian systems (see page 28) contributing to osteoporosis, or as a result of other diseases such as hyperparathyroidism

(51). Increased cortical porosity has been associated with an increased risk of fractures (52).

2.3.2.2. Trabecular architecture

Trabeculae are arranged within bones as struts, which connect with other trabeculae (see figure 3, page 26). The number of trabeculae which connect with each other (trabecular connectivity) is associated with elasticity (see below) and therefore trabecular connectivity contributes to fracture risk (53). The amount of trabecular connectivity generally reduces with age (54), and is reduced in osteoporosis, both in women (55) and men (56). Trabecular connectivity is also reduced with oral corticosteroid use (57) resulting in an increased risk of fractures.

2.3.3. Mechanical properties of bone

The main mechanical properties of bone that help resist fracture are elastic properties, particularly those related to energy absorption, and tensile strength. These are related by fracture mechanics. The mechanical property of bone depends on its material properties, macro-architectural structure and geometry. Most of the data on mechanical properties of bone have been obtained by the mechanical testing of small specimens of bone (25), and so are only influenced by material properties and macro-architecture. The other main technique is nanoindentation (58) which is similar to conventional hardness testing but performed on a much smaller scale. The force required to press a sharp diamond indenter into a material is measured as a function of indentation depth. As depth resolution is on the scale of nanometers, it is possible to conduct indentation experiments even on thin films.

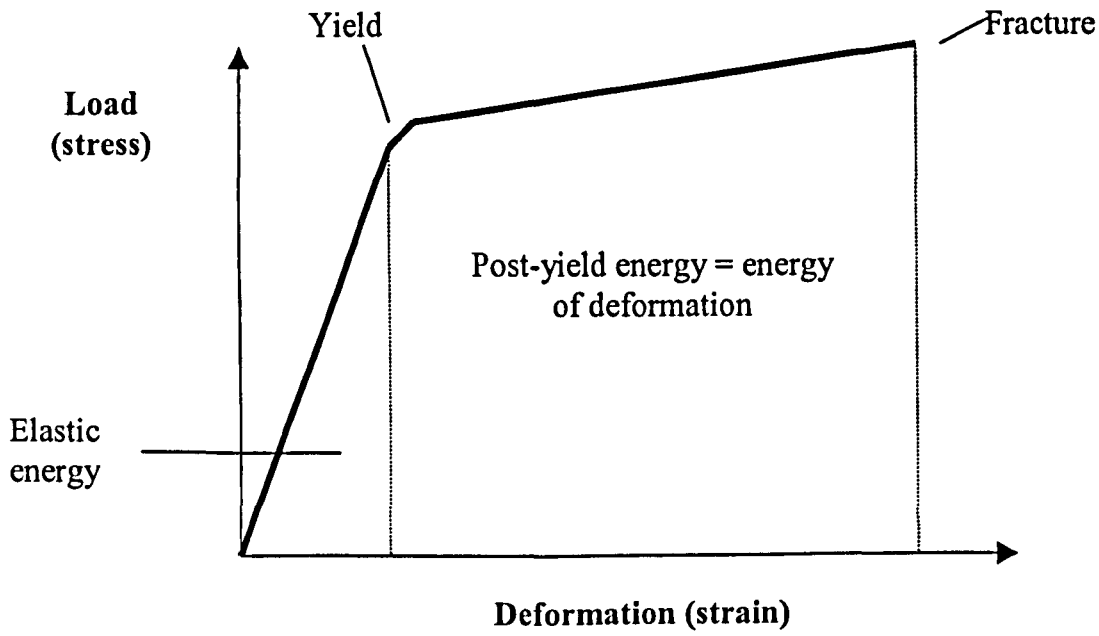
2.3.3.1. Elastic properties of bone

An elastic material returns to its original size and shape when loads are removed and the magnitude of the force needed to maintain a particular change in shape is governed by the material's stiffness. The most commonly quoted measure of stiffness is Young's modulus, defined as stress divided by strain in the linear region of a load-deformation curve.

The main way of measuring the elastic properties of bone is by applying a load to a specimen and calculating the elastic properties from the resulting deformations. In a mechanical test the load may be applied in tension (pulled apart) or compression (pushed together). Applying a load to a bone specimen will produce a tensile load-deformation curve, which can be arithmetically converted into a stress-strain curve (see Figure 5, below) given the dimensions of the specimen. This stress-strain curve will provide information on Young's Modulus and a description of the post-yield behaviour, which provides information on the toughness of the specimen.

Bone is slightly viscoelastic; that is its measured Young's modulus depends upon the rate at which the strain is applied and not all of the energy of deformation is returned when the load is removed (25). This means bone can flow somewhat when it is strained, but not indefinitely (59).

Figure 5: Load-deformation curve of a bone specimen loaded in tension. Adapted from Currey JD Bones: Structure and Mechanics, 2002 (25), page 34



The results of this mechanical method of measuring elastic properties of bone show considerable variation depending on the orientation of the bone specimen. The stiffness of bone measured along the length of the bone is about 1.6 to 2.4 times as great as that

measured at right angles to the length (60). This differing property of bone depending on the direction of measurement is called anisotropy.

Bones seem to be designed so that the forces they experience in everyday life usually load them into the elastic region only (25). This means they rarely pass the yield point where microdamage occurs, and rarely progress to fracture. Microdamage that occurs in bone during the post-yield phase comprises tiny cracks in the material, which can cause a permanent deformation. These cracks may heal spontaneously, perhaps by the process of remodelling with the formation of Haversian systems (39).

2.3.3.2. Tensile strength of bone

The strength of bone is the load a bone can bear before it breaks. Again, there are differences in the strength properties when bone is loaded in different directions (25). The tensile strength and strain to failure of bone is higher when it is loaded longitudinally rather than circumferentially. Bone is also stronger when loaded in compression than when loaded in tension.

2.3.3.3. Stiffness of bone

Bone is a composite material: it contains both collagen, hydroxyapatite mineral, and pores. The stiffness of bone arises from the properties of each of these materials, and from the way they interact. The more porous the bone the less stiff it will be and, in contrast, the more mineral the bone contains the stiffer it will be. Stiffness of bone material is one of the most important features, as the main function of the skeleton is to provide an inflexible structure (25). Stiffness is linked to toughness through energy absorption.

2.3.3.4. Fracture mechanics of bone

The fracture mechanics of bone are determined by the material properties of bone, macro-architecture and the geometry of bone. Geometry is covered on page 37.

The main aim of fracture mechanics is to predict fracture of an object based upon the energy of deformation and the toughness of the material. The energy of deformation is the area under the stress-strain curve in Figure 5, page 34. Elastic materials each have a characteristic fracture energy that is independent of the shape of the stress-strain curve.

If the bone is very stiff then the fracture energy can be achieved by very small deformations, whereas the skeleton needs a certain stiffness to function. In an impact not all of the energy that the bone absorbs causes deformation. Collagen can undergo a structural transition at body temperature that absorbs a large proportion of the energy and converts it to heat. Toughness is the ability of a material to resist fracture and can be measured by standard tests. At the tip of a crack the load is concentrated and the local stress is raised; this makes the material more likely to fracture further. In brittle materials this concentration is generally sufficient to cause a small crack to run throughout the material. If a material can counteract the load concentration by blunting the crack then the crack is less likely to propagate.

During loading, bone not only has mechanisms to reduce energy of deformation by structural transitions, but also can use small-scale fractures to its advantage. Micro-cracks appear in bone during loading and these have two effects: they can blunt cracks and they can reduce stiffness thus requiring larger deformations for full fracture. However too many micro-cracks may cause them to extend, spread and coalesce until the whole bone fractures in half. The rate at which these micro-cracks spread depends on the material properties of bone. The interaction of travelling cracks with the structure of bone is a complex process, and is not well understood.

Another type of micro-fracture that can occur in bone material is fatigue fracture. These micro-fractures are thought to occur due to repeated loading. A suggestion has been put forward that fatigue micro-fractures are caused by the results of damage to the osteocytes by transient ischaemia and reperfusion (61). From the material science point of view, in fatigue micro-fractures the volume of bone stressed is important. Large specimens will have more weak points than small specimens as each micro-fracture is a point of stress concentration.

2.3.3.5. Modelling the material properties of bone

Modelling of material properties of bone is needed, as there is no way at present to measure these properties in free-living humans. Material scientists have not yet developed a comprehensive framework for explaining the material properties of bone. The problem is to produce a model that incorporates all the important features: viscoelastic behaviour, the volume of mineral, anisotropy, and the detailed arrangement

of the microscopic and molecular constituents of bone. One reason why modelling does not give very good answers about the properties of bone is due to the mineral component. The mineral crystals are very small, but have a very high volume fraction, so that they distort the stress fields around themselves (25).

2.3.4. Structural geometry of bone

As well as bone mass (covered in the next section, page 41), the risk of fracture depends on the three-dimensional geometry of the bone under stress. Some consider that bone geometry is at least as important as bone density in fracture prediction (62). The geometrical measurements considered to be most important in fractures are linked to size (cross-sectional area, bone area, width and length) and bone shape (bending breaking resistance index, hip axis length) (25).

2.3.4.1. Size

Cross-sectional area of bones

Increased cross-sectional area of long bones is thought to reduce the risk of fracture. One study investigated the association between bone cross-sectional area and fracture risk in girls (19). In this study, girls with fractures had smaller cross-sectional areas at the distal radius ($P<0.001$). On average the cross-sectional area of the un-fractured radius in girls with forearm fractures was 8% smaller than in girls who had never fractured (19). A study carried out on 18 pairs of adult cadaveric forearm specimens showed that the cross-sectional area of the radius gave the best correlation with load to fracture with an r^2 of 0.80 calculated in a sequential multiple regression (63) compared to bone mineral density (BMD) or bone mineral content (BMC).

Bone area

In one study, metacarpal area in adult men, but not women, predicted hip fracture (64) suggesting this may be a surrogate measure for whole male skeletal geometry. In this study, 1386 women and 1014 men had their metacarpal area measured on hand radiographs, and were then followed up for 25 years as part of the Framingham Osteoporosis Study. In men, the age-adjusted risk of hip fracture was increased by 38% per standard deviation (SD) decrease in metacarpal area (hazard ratio 1.38, 95%CI 1.02

to 1.87). However, in women there was no increase in hip fracture according to metacarpal cortical area measurements. In a case control study of 200 boys with and without fractures, total body bone was smaller in children with fractures compared to those without (65).

Width and length of bones

In adults, increased width of bones has been shown to be associated with a reduced risk of fracture in some studies (66-68), but not all (69). One study reported that bone width relative to body mass predicts fractures (70), but not bone width alone. The length of long bones may also be associated with fractures: one study reported that greater leg length is associated with an increased risk of fractures (71). The association between bone size and fractures in children is discussed in more detail on page 133.

2.3.4.2. Shape

The shape of bones are important, as they influence fracture risk. Bone shape and its contribution to fracture risk can be measured by estimating the cross-sectional moment of inertia (CSMI), the section modulus (Z), the bending breaking resistance index (BBRI), and by detailed structural analysis of the hip.

Cross-sectional moment of inertia

Regional bone mass data of long bones collected by various methods (see page 44) can be used to calculate projected outer diameter, and the mean diameter of the medullary cavity. These values can be used to calculate the cross-sectional moment of inertia (CSMI). Moment of inertia is a measure of the cross-sectional area and how bone mass is distributed around the middle of the bone. It is an important index of structural rigidity (72), and when it is measured in the hip (72,73), tibia (74) or radius (75) it predicts fracture risk of that specific bone.

Section modulus

Section modulus (usually denoted by Z) can also be calculated from long bones and is a function of the moment of inertia and bone width and is a measure of how evenly bone

tissue is spread around the central axis of the bone. It has been shown to be related to the bending and torsional stiffness of intact tibias (25), and also fracture risk (49).

Bending breaking resistance index

Bending breaking resistance index (BBRI) is a component of the cross-sectional moment of inertia (62). The BBRI of the proximal radius has been shown to predict hip fracture in adults, although less accurately than BMD of the femoral neck measured by dual energy X-ray absorptiometry (DXA) (62,76), see page 45.

Structural analysis of the hip

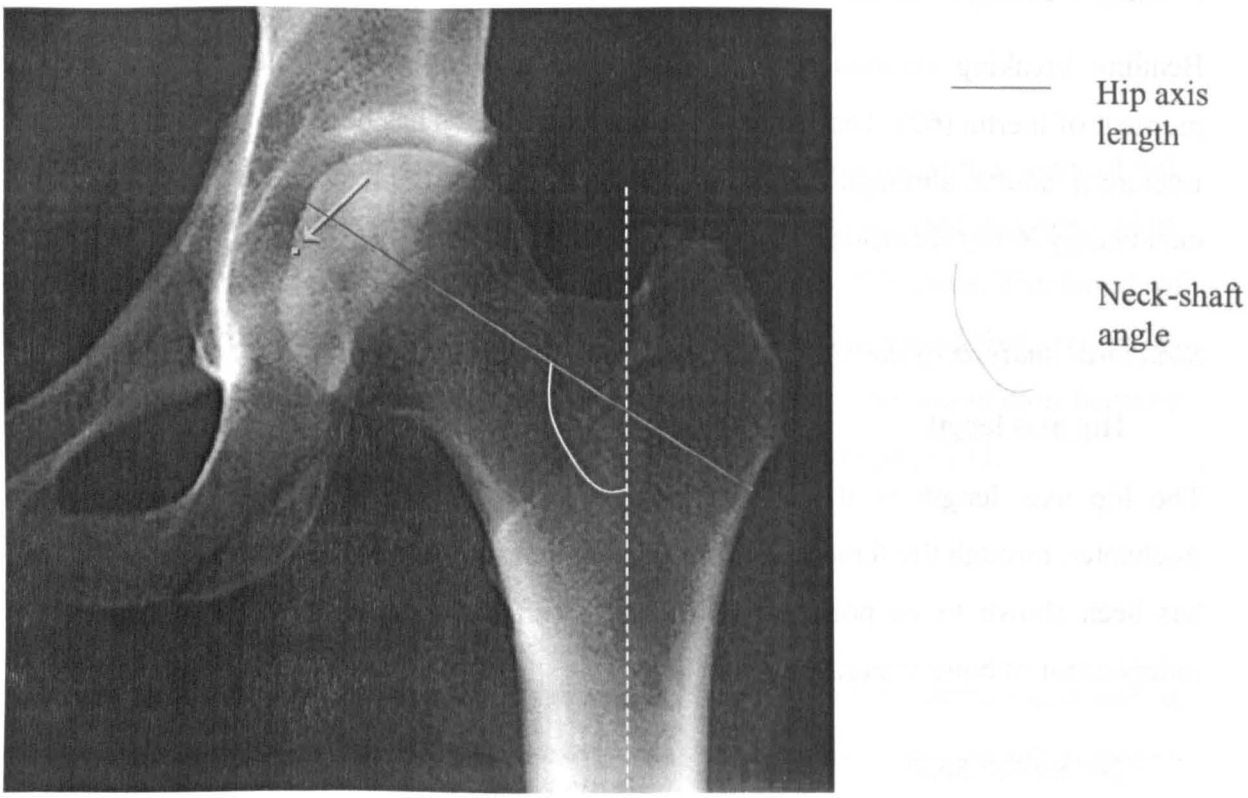
Hip axis length

The hip axis length is the distance from below the lateral aspect of the greater trochanter, through the femoral neck to the pelvic brim (77). See Figure 6, page 40. This has been shown to be positively associated with hip fractures in adults (77) and is independent of bone mineral density (78).

Neck-shaft angle

The measurement of the femoral neck-shaft angle in degrees is shown in Figure 6 page 40, and is the angle formed between the femoral neck axis and the femoral shaft axis. A greater angle has been associated with an increased risk of fractures in adults (69).

Figure 6: Diagram showing measurement of hip axis length from pelvic brim to below the greater trochanter, and neck-shaft angle



2.3.5. Summary

The ability of bones to resist fracture depends on their mass, material properties and geometry. The important materials of bone that contribute to strength are the collagen and mineral components. The macro-architectural structure of both the cortex and trabecular also contribute to bone strength. All of these factors along with overall bone geometry contribute to the mechanical properties of bone which help resist fracture: elasticity, which is partially governed by the bone's stiffness due to the presence of collagen, mineral and pores; and the tensile strength of bone which varies depending on the direction of loading. Fracture mechanics describes how breaks in bones start with micro-fractures that extend, spread and coalesce until the whole bone fractures in half. These micro-fractures interact with the structure and material properties of bone in a complex manner, and the micro-architectural structure of bone can blunt these micro-fractures and prevent spread.

2.4. BONE MASS AND OSTEOPOROSIS

This section is divided into four parts. The first discusses the distinction between bone mass, bone size and bone density and the second defines osteoporosis. The third part reviews techniques available for measuring bone mass and the last section focuses on measuring volumetric bone density, bone size and markers of bone turnover in children.

2.4.1. What is bone mass?

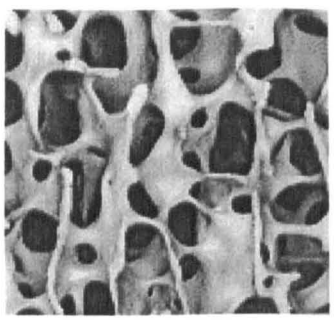
Bone mass is governed by the size of the skeletal envelope (bone size) and the density of bone mineral within (volumetric bone density). These two components of bone mass may have different determinants and for the rest of this thesis are considered separately where possible.

2.4.2. Definition of osteoporosis

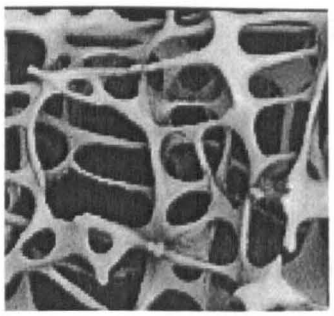
Osteoporosis is a progressive systemic skeletal disorder characterised by low bone mass and micro-architectural deterioration of bone tissue (see Figure 7, page 42) with a consequent increase in bone fragility and risk of fracture (79). Thirty percent of adult women living in high-income countries are likely to sustain an osteoporotic fracture in their lifetime, and osteoporotic fractures are more common with increasing age. From 1991 to 1992 the annual UK incidence of hip fractures was 30 per 100 000 in women aged 45 to 64 years, rising to 630 per 100 000 in women aged 75 to 85 years (9). For men, the corresponding incidence rates of hip fracture were 20 and 180 per 100 000. Osteoporotic fractures in the elderly currently cost the NHS approximately £1.5 billion per year (80). Osteoporosis is also becoming a global problem as life expectancy increases in low- and middle-income countries. Demographic shifts over the next 50 years will lead to huge increases in the number of elderly in Asia, South-America and Africa. Consequently, some 75% of hip fractures are expected to occur in low- and middle-income countries by 2050 (81).

Figure 7: Picture of normal and osteoporotic bone, showing the micro-architectural deterioration that occurs with osteoporosis. By kind permission of Alan Boyde. Image available at http://www.batsoc.org.uk/gallery/default.htm

(A) Normal bone



(B) Osteoporotic bone

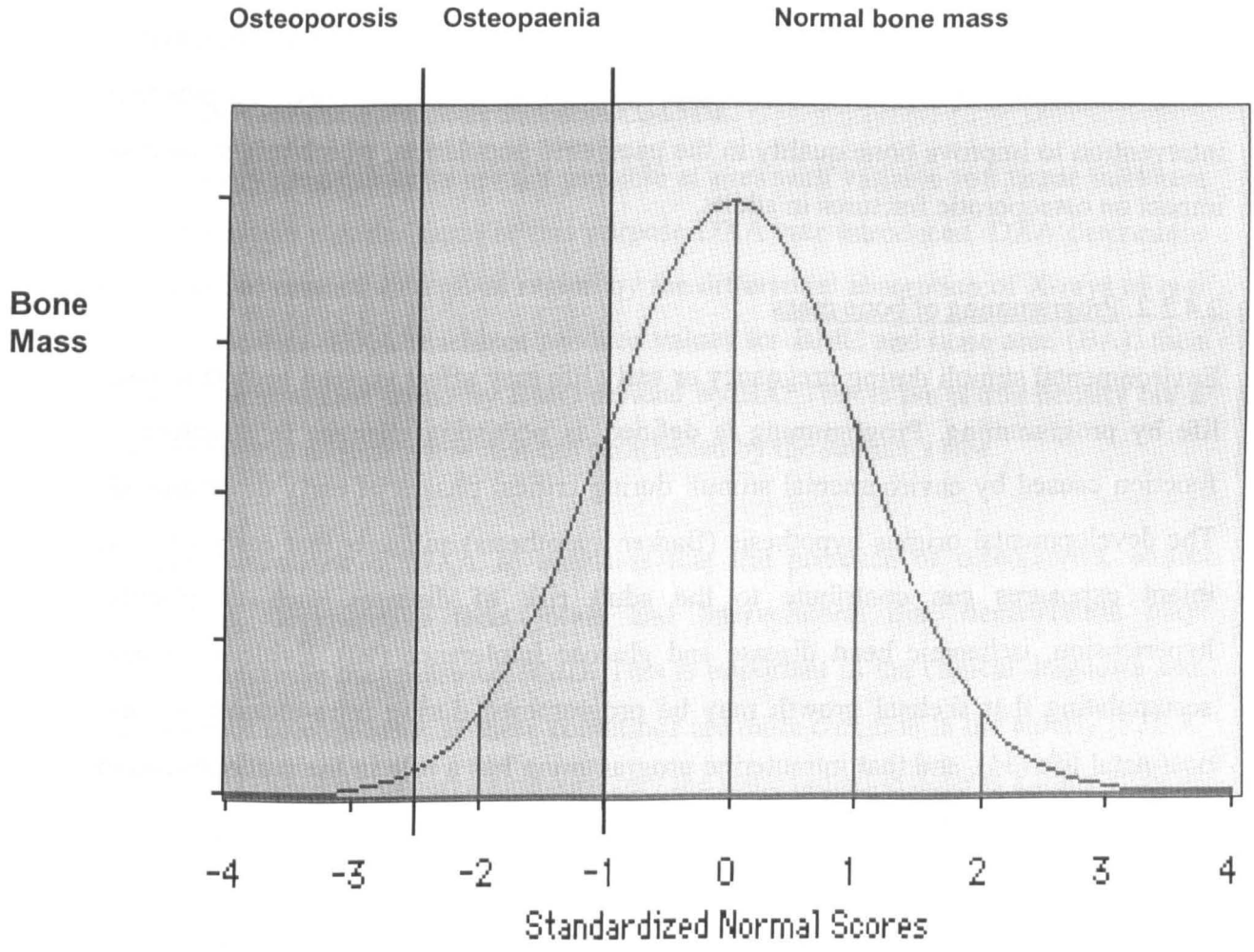


Osteoporosis can be defined in adults, as low bone mineral density (BMD) or bone mineral content (BMC) measured by a dual-energy X-ray absorptiometer (DXA) scan. BMD is a two dimensional representation of three-dimensional density, and is measured in g/cm^2. Adult BMD is a function of both bone size and density (82). Several methods have been suggested to adjust this data to get a better reflection of volumetric densities, including various regression methods of BMC on area (83). Normal BMC or BMD is defined by the World Health Organisation (WHO) as a value within one standard deviation of the young adult reference mean for that gender. Low bone mass, or osteopaenia is defined as a value for BMC or BMD more than one standard deviation below the young adult mean, but less than 2.5 standard deviations below this value. Osteoporosis is defined as a value for BMC or BMD 2.5 standard deviations or more below the young adult mean (see Figure 8, page 43). For adults, each standard deviation decrease in BMD approximately doubles fracture risk (81).

Osteoporosis may be idiopathic or secondary to other diseases. Idiopathic osteoporosis occurs as a result of the natural age related loss in bone mass. However, a substantial proportion of the variance in bone mass found in the general population cannot be explained by known genetic or environmental determinants (84). Most bone volume has accumulated by late adolescence, but bone mineral accrual proceeds throughout the third decade of life meaning that peak bone mass is usually reached by the late 20s (85). From then on, bone mass falls with age. Bone loss in women is relatively rapid in the 5 to 10 years following the menopause, which is why idiopathic osteoporosis is most

common in post-menopausal women. Osteoporosis therefore arises as a result of a low peak bone mass and / or excessive subsequent bone loss. The risk of fractures from osteoporosis would therefore be higher in an individual who starts with a lower peak bone mass, or who has excessive subsequent bone loss.

Figure 8: Distribution of bone mass in a population showing normal, osteopaenic and osteoporotic bone mass. Based on figures provided by the World Health Organisation (81).



2.4.2.1. Peak bone mass

Peak bone mass is the amount of bone tissue present when skeletal maturation is completed (86). There is evidence that the timing of peak bone mass is highly site specific with some regions acquiring peak bone mass earlier or later; for example peak bone mass occurs first in the femur (around aged 18 years) and later in the lumbar spine

and subcranial skeleton (around aged 22 and 24 years) (87). However, most bone mass will have been accumulated by late adolescence.

Although peak bone mass is an important determinant of the risk of osteoporotic fracture, little information is available about the determinants of peak bone mass. Among the potential determinants of peak bone mass are factors that cannot be influenced, such as genetic factors, and also factors that could be the target of interventions aimed at increasing bone mineral reserve. These include body composition (fat to muscle ratio), calcium intake and physical activity (88). However, genetic influences on adult bone size and mineral density may be modified by environmental factors such as undernutrition in-utero (89). Maximising peak bone mass is a potential intervention to improve bone quality in the paediatric population, which might have an impact on osteoporotic fractures in adults.

2.4.2.2. Programming of bone mass

Environmental stimuli during pregnancy or early life may affect skeletal growth in later life by programming. Programming is defined as persisting changes in structure or function caused by environmental stimuli during critical phases of early development. The developmental origins hypothesis (Barker hypothesis) suggests that early life and infant exposures can contribute to the adult risk of diseases such as obesity, hypertension, ischaemic heart disease and glucose intolerance (90). Evidence is also accumulating that skeletal growth may be programmed during intra-uterine or early post-natal life (34), and that intrauterine programming has a role in the pathogenesis of osteoporosis in adults (91).

2.4.3. Techniques for measuring bone mass

In adults, the current methods available to measure bone mass are single X-ray absorptiometry, dual X-ray absorptiometry (DXA), quantitative ultrasound (QUS), quantitative computed tomography (QCT), peripheral quantitative computed tomography (pQCT) and radiogrammetry. Low radiation dose, availability and ease of use have made DXA the most widely used technique for measuring bone density in

children. See Table 1 on page 47 for a summary of radiation doses for each of the techniques.

2.4.3.1. Single X-ray absorptiometry

Single X-ray absorptiometry makes a quantitative assessment of the BMC at peripheral sites of the skeleton. A small X-ray tube is used to measure radiation attenuation at the measurement site. Because single X-ray absorptiometry is a projectional technique, separate measurement of trabecular and cortical bone is not possible (92).

2.4.3.2. Dual energy X-ray absorptiometry (DXA)

Single energy measurements are not possible at sites with variable soft tissue thickness and composition e.g. the hip. For this purpose DXA was introduced. DXA determines the amount of mineral in a given region by the differential absorption of X-rays of two different energies. DXA machines produce values for BMC and bone area (BA), then calculate the so-called BMD by BMC divided by BA. This is not a true density but a two-dimensional measurement that can be affected by the subject's size.

The main drawback of DXA in adults is that the presence of osteophytes, aortic calcification, degenerative facet joints and intervertebral disc degeneration may artificially increase the measured BMD. This is important in the clinical diagnosis and assessment of osteoporosis, as these conditions are more common in the elderly (92). A whole body DXA scan imparts approximately the same radiation dose as a trans-atlantic flight (information from DXA manufacturers Lunar Prodigy).

2.4.3.3. Quantitative ultrasound (QUS)

Ultrasound measures the speed and attenuation of sound through bone and these measurements relate to bone density and architecture (92). The attractiveness of QUS lies in its low cost, portability, ease of use and freedom from ionising radiation. The correlation with BMD measured by DXA is at best moderate and the errors in predicting lumbar spine or femoral neck BMD are too high to allow QUS to be used to estimate BMD *per se* (93). Clinically, QUS is able to discriminate between populations with fractures and those without (94), despite the only moderate correlation with DXA. It is not entirely certain what QUS measures, but the speed of sound (SOS) through

bone measured by QUS (also called bone transmission time, BTT) is thought to be related to the elasticity of bone, cortical porosity and other structural measures such as cross-sectional area (95). Other measures of bone made by QUS include the apparent velocity of ultrasound (AVU) which correlates reasonably well with cancellous bone yield strength (96), and broadband ultrasound attenuation (BUA) which can also predict fractures (97).

2.4.3.4. Quantitative computed tomography (QCT)

QCT can provide a three-dimensional unobscured image of the bone, and accurately measures bone dimensions and distinguishes between cortical and trabecular bone. It can determine the true volumetric density of trabecular or cortical bone at any site. QCT has been principally employed to determine trabecular bone density in the vertebral column (98).

QCT can be performed in single energy (SEQCT) or dual-energy (DEQCT) modes, which differ in accuracy, precision and radiation (99). The accuracy of SEQCT is affected by the presence of fat, particularly fat in the marrow of bones (100). This causes SEQCT measurements to under-estimate BMD. It is possible to improve accuracy by using DEQCT, but this gives a higher dose of radiation and is only recommended for research purposes (92). QCT radiation doses are about 10 times that of DXA (101).

2.4.3.5. Peripheral quantitative computed tomography (pQCT)

Special purpose peripheral QCT (pQCT) scanners have been produced to measure BMD and BMC of the peripheral skeleton. pQCT allows for a true volumetric density measurement of appendicular bone without superimposition of other tissues, and provides exact three-dimensional localisation of the target volume (92). Relatively low doses of radiation are used.

2.4.3.6. Radiogrammetry

In radiogrammetry, measurements are made on plain X-rays, usually of hands when it is called metacarpal morphometry. In metacarpal morphometry the measurements are made at the midshaft of the right second metacarpal. The length of this bone is measured with a millimetre rule or needle-tipped Vernier callipers, as is the outer

diameter or periosteal width, and the inner diameter or medullary space. The cortical area is then calculated. Though this is an indirect measure of bone mass, it has been shown to correlate with the ash content of the bone (102). The radiation dose is lower than QCT, but significantly higher than DXA scans. The Pronosco system is a method of estimating bone density from hand radiographs (103) and this is strongly correlated with DXA-measured BMD of the wrist (correlation coefficient 0.90), but less strongly with DXA-measured BMD of the hip (correlation coefficient 0.61).

2.4.3.7. Magnetic resonance imaging (MRI)

MRI uses strong magnetic fields to vibrate the nucleus of hydrogen atoms within water molecules. The scanner picks up these vibrations and a digital three-dimensional image is produced. No ionising radiation is involved. MRI is currently a research tool used for structural characterisation of trabecular bone in people with and without osteoporosis (104). It can also be used to assess vertebral marrow perfusion and the amount of vertebral marrow fat (105).

Table 1: Doses of radiation measured in mili-Sieverts (mSv) for various techniques available to measure bone mass, compared to average background radiation for a person in the UK per year and a transatlantic flight.

Radiation source	mSv	per	Reference
Background radiation in UK	2.7	year	(106)
Transatlantic flight	0.0375	flight	(107)
CXR	0.02	X-ray	(108)
L spine X-ray	2.4	X-ray	(108)
Total body DXA scan	0.05	scan	(109)
QUS	none		
QCT	1 to 10 (depending on mode)	scan	(110)
pQCT	0.02	scan	(111)
Radiogrammetry	0.1 to 1	X-ray	(110)
MRI	none		

Abbreviations: CXR chest X-ray; DXA dual energy X-ray absorptiometry; L lumbar; MRI magnetic resonance imaging; mSv mili-Sieverts; pQCT peripheral quantitative computed tomography; QCT quantitative computed tomography; QUS quantitative ultrasound; UK United Kingdom

2.4.4. Measuring volumetric bone density in children

2.4.4.1. Using DXA

DXA is the most common technique in children, because it is widely available, results in a modest dose of radiation and is relatively cheap. DXA scans can also be used to obtain a measure of fat and lean mass. However, DXA scans can only produce a value for *estimated* volumetric bone density: volumetric bone density cannot be measured directly using DXA because DXA measurements are based on the two-dimensional projection of a three-dimensional structure, and they are therefore a function of both the mass and size of the bone being examined (see section on DXA starting on page 45). Simply measuring BMC or BMD means than any association studies are likely to be confounded by body size. Although the problem of size in bone densitometry is well appreciated, there is no consensus on the most appropriate way to correct results for size to obtain an estimate of volumetric bone density. A number of different approaches have been suggested:

1. The use of multiple regression analysis simultaneously to adjust BMC for BA, weight, height and other relevant factors such as age, pubertal status (112-114)
2. The use of calculated volumetric bone density (e.g. bone mineral apparent density, BMAD) in which BMC is adjusted for calculated bone volume rather than bone area (115).

Another important consideration is the influence of head BMC on the results for total body BMC, as the contribution of head BMC to measured total body BMC is as much as 51% in children aged 4.5 to 5.5 years (116), but contributes little to fracture risk. Because of this, it has been suggested that using size-adjusted total body minus head BMC or BMD as estimates of volumetric bone density is likely to result in more predictive power (117). Motion artefacts are another limitation of DXA studies in the very young and in uncooperative children as movement can affect alignment (118).

2.4.4.2. Using other methods

pQCT can directly measure volumetric bone in children (see section on pQCT starting on page 46), but is not used as frequently as DXA because of expense and a slightly higher radiation dose. In early studies on children single energy X-ray absorptiometry

(see section on page 45) has been used, but this also produces an estimated volumetric density and will need to be corrected for body size. Radiogrammetry (page 46) has also been used in children, but usually in early studies before DXA scanners became widely available. QUS does not produce measures of either estimated or measured volumetric density, but measures such as BTT or AVU which have been correlated with types of material properties of bone.

2.4.5. Measuring bone size in children

Bone size can be measured in children more easily than volumetric bone density. Bone area measured by DXA is a two-dimensional measurement (in cm^2), and can be of the whole skeleton or specific regions such as upper or lower limbs. Length or width of bones can theoretically be measured by DXA via the use of 'Regions of Interest', but it is not commonplace to do this. pQCT of femur, tibia and fibula or distal forearm can be used to accurately measure not only cross-sectional area (CSA) of long-bones, but to also measure precisely cortical thickness. QCT of the lumbar spine can be used to measure vertebral height or vertebral CSA. Radiogrammetry of the hands can be used to measure cortical thickness and bone length.

2.4.6. Other measures of bone status in childhood: bone turnover markers

Bone remodelling is the result of two opposite activities (see page 28), the production of new bone matrix by osteoblasts and the destruction of old bone by osteoclasts. The rates of bone formation and bone resorption can be evaluated by measuring predominately osteoblastic or osteoclastic enzyme activities, or by assaying bone matrix components released in the bloodstream and excreted in the urine. See Table 2, page 50 for a summary of the markers currently available.

Table 2: Biochemical markers of bone turnover with their abbreviations in brackets. Adapted from Garnero et al (119)

Markers of bone formation	Markers of bone resorption
· Bone specific alkaline phosphatase (bone ALP or BAP) · Osteocalcin (OC) · Type I collagen extension propeptides (PICP, PINP)	Serum/plasma markers · N-terminal (S-NTX) and C-terminal (S-CTX) cross-linking telopeptide of type I collagen · Tartrate resistant acid phosphatase (TRACP, 5b isoenzyme) Urine markers · Deoxypyridinoline (DPD) · Pyridinoline (PYD) · Galactosyl-hydroxylysine (Gal-Hyl) · Hydroxyproline (Hyp or HPro) · Type I collagen helicoidal peptide 620-623 · N-terminal (U-NTX) and C-terminal (U-CTX) cross-linking telopeptide of type I collagen

2.4.6.1. Markers of bone formation

The precise function of the bone isoform of alkaline phosphatase (BAP) is not known. It is one of a group of enzymes abundant in many tissues (119). Osteocalcin is one of the non-collagenous proteins of bone (see section on NCPs, page 23). The two procollagen type I extension propeptides are cleaved from procollagen I in the extracellular space and liberated into the circulation, and are considered quantitative measures of newly formed type I collagen (120). They are aminoterminal propeptide (PINP) which circulates in blood as a trimer, and carboxyterminal propeptide (PICP) which is a single globular trimeric molecule.

2.4.6.2. Markers of bone resorption

The majority of markers of bone resorption (except tartrate resistant acid phosphatase) are products of bone collagen degradation. They can be measured in either the serum/plasma or the urine.

Serum/plasma markers of bone resorption

The N aminoterminal telopeptide of type I collagen (NTX) can be measured in either the serum (S-NTX) or the urine (U-NTX) (120). It is not completely specific for bone as it is also released from skin collagen. The C carboxyterminal telopeptide of type I collagen (CTX) can also be measured in either the serum (S-CTX) or urine (U-CTX). It is present in several forms as it undergoes post-translational modification with ageing (121). Tartrate-resistant acid phosphatase (TRACP) belongs to a group of acid phosphatases and the isoform TRACP-5b is characteristic of osteoclasts.

Urine markers of bone resorption

Hydroxypyridinium cross-links of collagen (pyridinoline - PYD, and deoxypyridinoline - DPD) are bridge molecules formed during the extracellular maturation of fibrillar collagen that stabilize the extracellular matrix of bone (120). Hydroxyproline (Hyp or HPro) is released from mature bone collagen during bone resorption (121). However, it is not specific to bone resorption as it is also released from catabolized extension propeptides of procollagen type I during bone formation, from non-bone collagen and also comes from dietary components (120). Galactosyl-hydroxylysine is a better marker of bone resorption than hydroxyproline as it is fairly specific for bone and is not influenced by dietary components, but the major disadvantage is that there is not a convenient immunoassay (120).

2.4.7. Summary

Bone mass is governed by the size of the skeletal envelope (bone size) and the density of bone mineral within (volumetric bone density). In adults, a bone mass two-and-a-half standard deviations below the mean is described as osteoporosis and is associated with an increased risk of fractures. Many techniques are available to measure bone mass. In children size-adjusted DXA measures are the most common method of estimating volumetric bone density. Bone size in children can be measured by various techniques and bone turnover markers are available to give information on bone formation and bone resorption.

2.5. SUMMARY OF BONE CHAPTER

- Bone is a composite material with a structural hierarchy
- Bone develops during embryonic life by intramembranous ossification or endochondral ossification
- During childhood and adolescence bones grow by modelling
- In adults bones are constantly remodelled by the formation of Haversian systems
- The ability of bones to resist fracture depends on their mass, material properties and geometry
- Bone mass is governed by both the size of bones and the actual volumetric density of bone mineral within
- The most common technique for measuring bone density in children is by dual energy X-ray absorptiometry (DXA) adjusted for body size (estimated volumetric bone density)
- Many techniques are available for measuring bone size and bone turnover in childhood

CHAPTER 3: DETERMINANTS OF BONE MASS IN CHILDHOOD

The previous chapter has discussed the structure and growth of bone and how bone mass can be measured in children. This chapter presents the descriptive epidemiology of bone mass in childhood and then focuses on the available literature on the determinants of bone mass in children.

The review was carried out in a systematic manner. In the section on determinants of bone mass in childhood I have divided bone mass into measures of volumetric bone density (estimated if measured by DXA, or measured directly if by CT), measures of bone size, and other measures of bone status as discussed on page 49. For volumetric bone density the search terms used were bone mass, bone density, bone mineral density, bone mineral content, bone mineral apparent density, volumetric bone density, true bone density and their abbreviations. For bone size the search terms used were cortical thickness, endocortical circumference, periosteal circumference, bone geometry, bone size, bone area, bone cross-sectional area, bone length, bone width, bone circumference, bone shape and their abbreviations. Studies which used DXA measures of bone mass not corrected for size are not discussed, as these outcome measures are likely to be confounded by body size (see previous chapter, page 48).

3.1. DESCRIPTIVE EPIDEMIOLOGY OF BONE MASS IN CHILDHOOD

3.1.1. Reference data on bone mass in childhood

There are a few papers (122-127) that present reference values for various measures of bone mass in small groups of children of various ages. Unlike adult populations, there are currently no standardised reference populations for childhood measures of bone mass that can be used with DXA machine software. The populations from which these reference values come from are geographically widespread. All use small numbers of children for each age group, but the reference ranges produced are similar across the studies. See Figures 9 and 10 (page 55) for the reference ranges for total body BMD and lumbar spine BMD separately for 188 boys and 256 girls aged 4 to 25 years from a study of children and young adults in Rotterdam.

3.1.2. Age

As can be seen from the Figures on page 55, all measures of bone mass increase with age. This is a consistent finding across all studies. There also seems to be an acceleration in bone mass accumulation around age 11 to 15 for girls and 13 to 17 for boys. This can be easily seen in the lumbar spine. Again, this is a consistent finding.

Figure 9: Lumbar spine BMD for 256 girls and 188 boys from Rotterdam, published in 2002 (126)

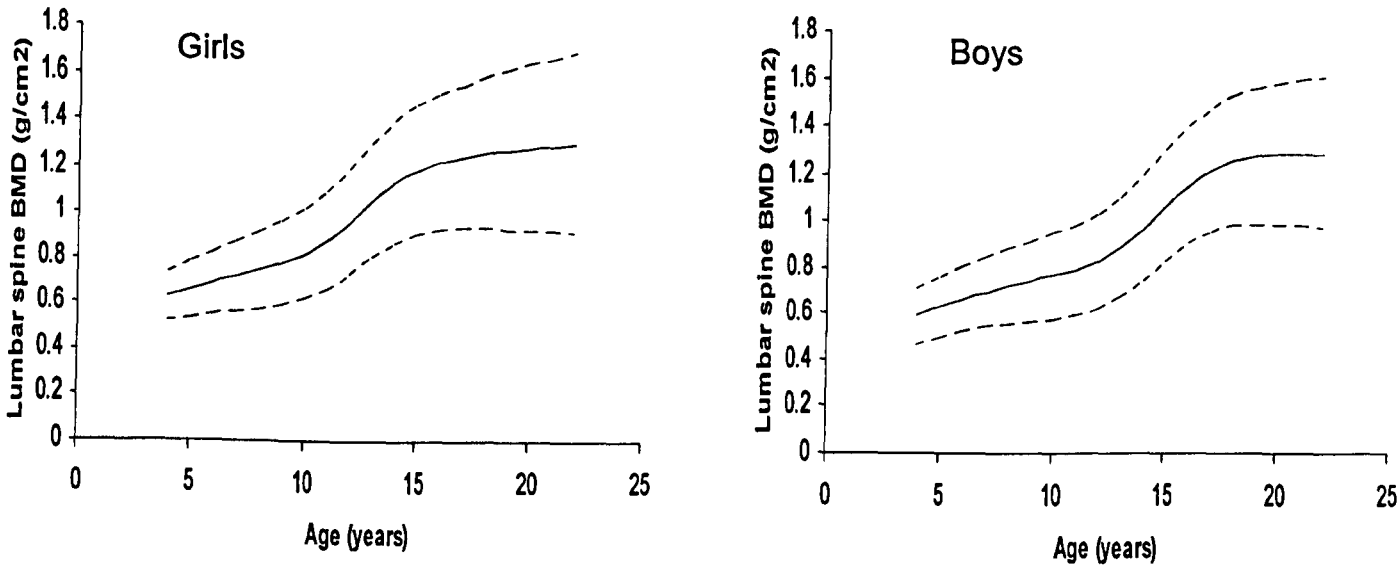
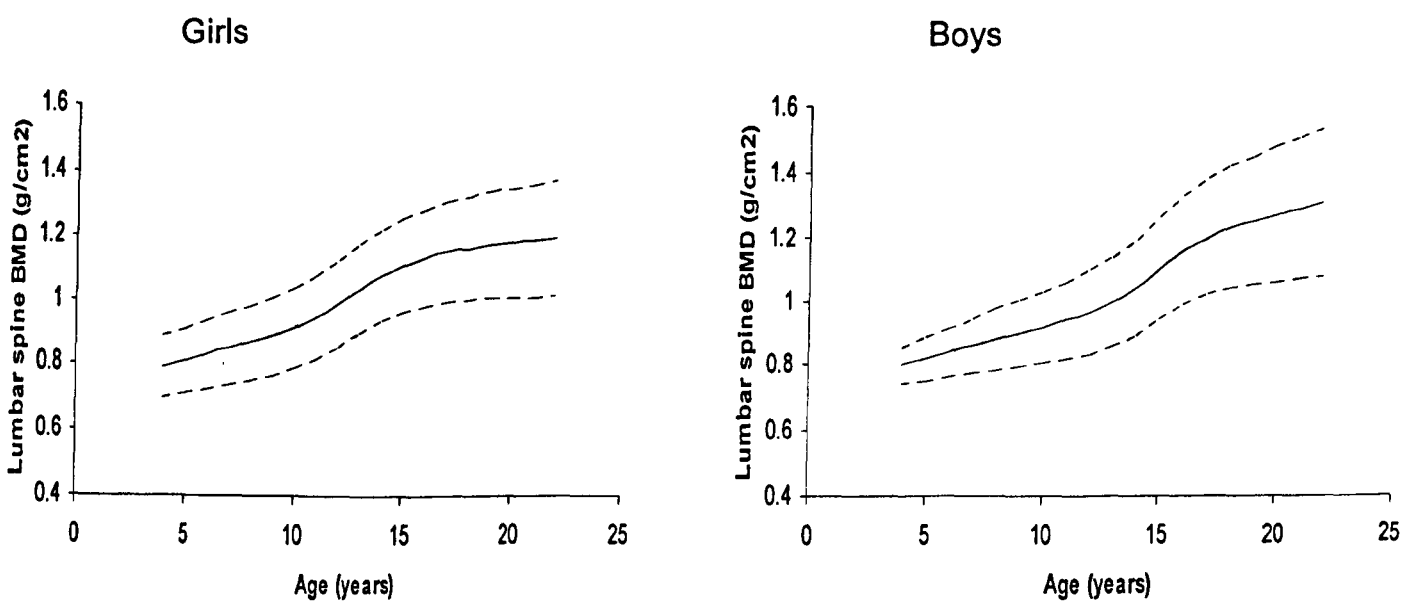


Figure 10: Total body BMD for 256 girls and 188 boys from Rotterdam, published in 2002 (126)



Abbreviations: BMD bone mineral density

3.1.3. Gender

The literature on the gender differences in bone mass in childhood is contradictory, and is summarised in Table 3 starting on page 57. Four studies (three using DXA and one using pQCT) report no gender differences in volumetric bone density in prepubertal children (128-131) and three studies (two using pQCT and one radiogrammetry) showed no difference at any age from infancy to young adulthood (132-134). However, two studies on 8-year old children, both using DXA, reported higher estimated volumetric bone density in boys than girls (135,136), and one study reports higher estimated volumetric density of the lumbar spine in prepubertal girls compared to boys (127).

In terms of bone size, three studies (two using DXA and one pQCT) report no prepubertal gender differences in bone size (128,130,131). Two studies using pQCT report no gender difference in vertebral body height but found vertebral CSA to be higher in boys (134,137). Two studies report greater bone size in boys after puberty (132,133), one using pQCT and one radiogrammetry.

A summary suggested by two review papers (138,139) is that volumetric bone density probably does not show important gender differences in childhood and adolescence, but boys have larger sized bones, particularly after puberty.

3.1.4. Ethnicity

In general, although there are many studies that have looked for ethnic differences in childhood bone mass, only some have taken into account ethnic differences in body size. Of those studies that have, the general conclusion seems to be that black children have a greater volumetric bone density than white children of similar developmental stages (136,140-145). One study using DXA measures reported no differences in estimated volumetric bone density between black and white children (128). Only four studies were found that investigated ethnic differences in bone size (128,141,142,146), and the results are contradictory so no conclusions can be drawn.

Table 3: Summary of studies investigating gender differences in bone mass in childhood

Author, year of publication	Age of participants	No in study	Measure of volumetric bone density used	Measure of bone size used	Other measures of bone status	Result
Specker et al, 1987, USA (130)	1 to 6 years	89	size-adjusted BMC of distal radius by DXA	width of distal radius by DXA		No gender difference in estimated volumetric bone density for children aged 12 to 35 months, or 36 to 59 months. Children aged 60-83 months showed boys had a higher BMC compared to girls (0.332 g/cm ± 0.064 vs 0.282 ± 0.046) but no difference in width at any age
Gilsanz et al, 1994, USA (132)	4-20 years	196	cancellous and cortical vertebral bone density by qCT	height and CSA of vertebral bodies by qCT		No gender differences at any age in cancellous or cortical density. Greater dimensions of vertebral bodies in males compared with females at all Tanner stages
Zamberlan et al, 1995, Italy (133)	3-17 years	325	size-adjusted volumetric BMC of radius by radiogrammetry	outer diameter, inner diameter, length and width of second metacarpal by radiogrammetry		No difference in volumetric bone density between males and females at any age. Higher outer diameter, inner diameter, length and width of second metacarpal in males after puberty only (actual values not given)
Gilsanz et al, 1997, USA (137)	8-13 years	60		height and CSA of L vertebral bodies, and CSA and cortical bone area of midshaft of femur by qCT	serum osteocalcin	CSA was 11% smaller in girls than boys ($P<0.0001$). No gender difference in heights of vertebral bodies; no gender differences in any measure of size at the femur; no gender differences in osteocalcin
Jones et al, 1998, Tasmania (135)	8 years	330	Size-adjusted hip BMD and size-adjusted L spine BMD by DXA			Boys had higher estimated hip volumetric density than girls (9.6% higher, 95%CI 6.9 to14%), but girls had a higher estimated L spine density than boys (3.2% higher, 95%CI 0.8 to 5.6%)
Unal et al, 2000, Turkey (129)	1,2,4,6,9 and 12 months	164	Size-adjusted TB BMC, TB BMD and L spine BMD by DXA			No gender difference in estimated volumetric bone density: e.g. for aged 12 months TB BMC in males 214 ± 16 and for females 197.8 ± 25
Koo et al, 2000, USA (128)	mean age of 2.1 days	201	L spine BMC and BMD by DXA (unknown if size-adjusted)	L spine bone area by DXA		No gender differences in either measure (actual values not given)

Table 3, continued

Author, year of publication	Age of participants	No in study	Measure of volumetric bone density used	Measure of bone size used	Other measures of bone status	Result
Horlick et al, 2000, USA (136)	8 years	336	Size-adjusted TB BMC by DXA			Greater in boys than girls by 14g ± 5.7, P=0.01
Arfai et al, 2002, USA (134)	5-10 years	62	body height and weight adjusted cancellous density of vertebrae by qCT	body height and weight-adjusted vertebral CSA and height by qCT		No gender difference in vertebral cancellous density (156mg/cm3 ± 26 for boys vs 152 ± 21), no difference in vertebral height (17mm ± 1.5 for boys vs 17 ± 1.8). Boys had greater CSA (663mm2 ± 114 for boys vs 565 ± 21, P<0.001)
Schoenau et al, 2002, Germany (131)	prepubertal (Tanner stage 1)	108	size-adjusted cortical density of radius using pQCT	cortical thickness of radius using pQCT		No prepubertal gender differences seen in either measured cortical density (998mg/cm^3 ± 47 in boys vs 981 ± 54) or cortical thickness (1.58mm ± 0.31 in boys vs 1.44 ± 0.41)
Fares et al, 2003, Lebanon (147)	10-17 years	172			biochemical markers of bone turnover: osteocalcin, bone alkaline phosphatase, S-CTX	After adjusting for age, weight and tanner stage boys had higher turnover markers than girls
Arabi et al, 2004, Lebanon (127)	10-17 years	363	size-adjusted L spine and hip BMD by DXA			Girls had greater estimated volumetric density of L spine before puberty (0.088 g/cm3 ± 0.009 vs 0.083 ± 0.006) but no difference seen at aged 17 years for L spine, and no difference in hip seen at any age
Vignolo et al, 2005, Italy (148)	in-utero	80			ultrasound of foetal spine to look for ossification centres	Ossification timing was earlier in females than males, P=0.019
De Paulo et al, 2005, Italy (149)	less than 3 days	200			BTT of humerus by QUS	BTT was significantly higher in males (actual results not given)

Abbreviations: BMC bone mineral content; BTT bone transmission time; CSA cross-sectional area; DXA dual energy X-ray absorptiometry; L lumbar; pQCT peripheral quantitative computed tomography; qCT quantitative computed tomography; QUS quantitative ultrasound; S-CTX serum C-terminal telopeptide of type I collagen crosslinks; TB BMC total body bone mineral content; TB BMD total body bone mineral density; USA United States of America

3.1.5. Summary of the descriptive epidemiology of bone mass in childhood

Bone mass in childhood increases with age, with an acceleration in accumulation between ages 11 and 15 years in girls, and between 13 and 17 years in boys. There is little gender difference in volumetric bone density in children, but boys have bigger sized bones. Black children have a greater volumetric bone density than white children of similar developmental stages. There is no data available on temporal differences in bone mass in children.

3.2. ANALYTICAL EPIDEMIOLOGY OF THE DETERMINANTS OF BONE MASS IN CHILDHOOD

As already discussed in the section on Bone Mass and Osteoporosis starting on page 41, peak bone mass is the amount of bone tissue present when skeletal maturation is completed, and most bone mass will be accumulated by late adolescence (86).

The determinants of peak bone mass can be categorised into factors acting during intra-uterine life, early post-natal life, through the years of growth and into young adulthood. I have divided these potential determinants of childhood bone mass (see Table below) into factors that are non-modifiable such as genetic factors; early life determinants such as maternal nutrition during pregnancy or birth weight; and those potentially modifiable determinants that could be the target of interventions such as physical activity. This is an arbitrary categorisation, as some variables could be in more than one category. For example, obesity is a determinant with composite genetic and environmental influences, but is also potentially modifiable.

Table 4: Categorisation of potential determinants of bone mass in childhood reviewed in this thesis

Determinants that are non-modifiable	Early life determinants	Other determinants that are potentially modifiable
·age ·gender ·genetic determinants ·ethnicity ·parental size ·pubertal stage ·hormonal / endocrine influences	·intra-uterine maternal nutrition maternal smoking maternal activity maternal illness season of birth gestational age birth weight/length ·early post-natal life breast feeding vitamin D status	·diet and nutrition in later childhood ·socio-economic status ·body composition ·physical activity ·sunlight exposure ·drugs and medications

There are also other factors relating to concomitant diseases, particularly those chronic diseases that results in paralysis, inflammation and immunosupression.

3.2.1. Non-modifiable determinants of bone mass in childhood

Age, gender and ethnicity have been discussed in the previous section starting on page 54.

3.2.1.1. Genetic determinants

There is increasing evidence that genetic factors play a role in bone accrual in childhood and adolescence. Two studies have been carried out that assess the association between mothers bone mass and that of their prepubertal offspring (150,151), and heritability estimates range from 18-51%. It is likely that the genetic component of bone mass in childhood is a combination of polygenic effects, gene-gene interactions and gene-environment interactions. It is therefore unlikely that a single genetic locus will by itself be the major determinant of childhood bone mass. Caution also needs to be used when interpreting the results of genetic analysis studies. Often, when a genetic polymorphism has found to be associated with a complex health outcome in one study, a similar association has not been found in other studies or populations. This inability to replicate many genetic associations may be due to inadequate sample sizes, failure to attribute results to chance, publication bias, confounding from population structure, misclassification of outcome, or true heterogeneity in gene-disease associations (152).

For this section I have divided the potential genetic determinants of bone mass in childhood into polymorphisms of genes that code for receptors, hormones/cytokines or bone matrix proteins. For the purposes of this thesis I have explicitly discussed the evidence available for the association between polymorphisms in the gene that codes for the vitamin D receptor and the oestrogen receptor alpha with childhood volumetric bone density or bone size, as multiple studies have been found that investigated these areas. All other polymorphisms have been investigated by single studies, some with small numbers, and their results are summarised in Table 5 on page 62.

Table 5: Summary of a literature review of the potential genetic determinants of bone mass in childhood.

Gene	Number of studies found (Reference)	Combined number of participants	Summary of results
Genes that code for receptors			
Vitamin D	8 (see text)	1506	5 found an association (some in girls only), 3 found no association
Oestrogen receptor alpha	5 (see text)	1035	2 report an association, 2 do not, 1 suggests the polymorphism modulates the association between physical activity and bone mass in girls
Androgen receptor	1 (153)	428	No association found with estimated volumetric density by DXA
Calcium sensing receptor	1 (154)	125	No association found with estimated volumetric density by DXA
β_3 adrenergic receptor	1 (154)	125	No association found with estimated volumetric density by DXA
Osteoprotegerin	1 (155)	124	Association found for estimated volumetric density of the lumbar spine by DXA
Genes that code for hormones/cytokines			
Parathyroid hormone	1 (154)	125	Association found for estimated volumetric density of lumbar spine but not femoral neck by DXA
Insulin-like Growth Factor 1	1 (156)	39	Association found with rate of increase in estimated volumetric density of the forearm by DXA
Genes that code for bone matrix proteins			
COL1A1	2 (153,157)	537	One found no association with estimated volumetric density by DXA, the other (157) found an association with measured trabecular density of vertebrae by CT, but no association with bone size
COL1A2	1 (153)	192	Association found for estimated volumetric density of total body by DXA
Osteocalcin	1 (153)	426	Association found for estimated volumetric density of hip by DXA

Abbreviations: BMC bone mineral content; BMD bone mineral density; COL1A1 collagen type 1 alpha-1; COL1A2 collagen type 1 alpha-2; CT computed tomography; ERα oestrogen receptor alpha; LRP5 LDL receptor-related protein 5; SNPs sequence nucleotide polymorphisms

Vitamin D receptor (VDR)

The vitamin D receptor (VDR) mediates the effect of the active form of vitamin D (1,25-hydroxyvitamin D_3) which is required for regulation of calcium metabolism along with parathyroid hormone (PTH). The VDR gene maps to chromosome 12q13-14 and several sites of sequence variation in the VDR gene have been described. There are a cluster of sites near exon 9 and the 3'-untranslated region (3'-UTR) and are detected by *BsmI*, *ApaI* and *TaqI* as restriction fragment length polymorphisms; there is a sequence variation detected at the translation initiation site detected by *TI*; and a sequence variation in the start codon detected by *FokI*. Eight studies were found which investigated the association between polymorphisms of the VDR and childhood volumetric bone density or bone size (153,154,156,158-162) (see Table 6, page 64). Results are inconsistent across studies, and vary depending on which measure of bone density has been used and which gender the children were. No conclusions can be drawn from the current studies on the association between polymorphisms of the VDR and either volumetric bone density or bone size in childhood.

Table 6: Summary of the main results of nine studies investigating the association between the vitamin D receptor gene polymorphisms and bone mass in childhood

	Vitamin D receptor gene polymorphism				
Author and participants	***FokI***	***ApaI***	***BsmI***	***TaqI***	***TI***
Sainz et al, 1997: 100 girls aged 6 to 12 years (158)		Association with measured volumetric bone density[a] found. No association with bone size[b] (girls)	Association with measured volumetric bone density[a] found. No association with bone size[b] (girls)	No association found with measured volumetric bone density[a] or bone size[b] (girls)	
Tao et al, 1998: 114 boys and girls aged 7 years (163)				Association with estimated volumetric bone density[c] found in girls only	
Laaksonen et al, 2004: 124 boys and girls aged 14 to 16 years (159)	Association with estimated volumetric bone density[d] found in boys only		No association with estimated volumetric bone density[d] found		
Backstrom et al, 2001: 39 children who were studied at aged 3 months and then again when aged 9 to 11 years (156)	Association with estimated volumetric bone density[d] found at age 9 to 11 years only	Association with estimated volumetric bone density[d] found at age 3 months only		Association with estimated volumetric bone density[d] found at age 3 months only	
Ferrari et al, 1998: 155 girls aged 7 to 12 years (160)	No association with estimated volumetric bone density[c,e] found (girls)	Association with estimated volumetric bone density[c,e] found (girls)	No association found with estimated volumetric bone density[c,e]		

Table 6, continued

Author and participants	Vitamin D receptor gene polymorphism				
	FokI	*ApaI*	*BsmI*	*TaqI*	*TI*
van der Sluis et al, 2003: 148 boys and girls aged 4 to 20 years (161)		No association with estimated volumetric bone density[c] found. Association found with bone size[f]	No association found with estimated volumetric bone density[c] Association found with bone size[f]		
Willing et al, 2003: 428 boys and girls aged 4 to 6 years (153)			No association with estimated volumetric bone density[c,e,g] found		No association with estimated volumetric bone density[c,e,g] found
Gunnes et al, 1997: 273 boys and girls aged 12 years (162)			No association with estimated volumetric bone density[c,d,e,g] found		

a cancellous vertebral bone density and cortical density of femur by qCT (measured volumetric bone density)
b CSA L vertebrae, CSA and cortical area of femur
c size-adjusted BMC of L spine by DXA (estimated volumetric bone density)
d size-adjusted BMC of radius by DXA (estimated volumetric bone density)
e size-adjusted BMC of femur by DXA (estimated volumetric bone density)
f mean width of L vertebrae by DXA
g size-adjusted TB BMC by DXA (estimated volumetric bone density)

Abbreviations: BMC bone mineral content; CSA cross-sectional areal; DXA dual energy X-ray absorptiometry; L lumbar; qCT quantitative computed tomography; TB BMC total body bone mineral content

Oestrogen receptor alpha (ERα)

Oestrogens play an important role in regulating skeletal growth and maintenance of bone mass in both males and females. They exert their effect through the oestrogen receptor alpha (ERα), a steroid hormone receptor that functions as a hormone-regulated transcription factor controlling the expression of specific target genes.

The ERα gene maps onto chromosome 6q25, and there are two polymorphisms in the first intron that can be detected by the restriction enzymes *PvuII* and *Xbal*. There is also a 5'-TA repeat polymorphism. Four studies were found that investigated the association between polymorphisms in the ERα gene and bone mass in childhood (153,164-166). Again, the results are inconsistent with two studies showing an association with estimated volumetric density (165,166), one showing no association (153) with estimated volumetric bone density, and one suggesting that the *PvuII* ERα polymorphism modulates the association between physical activity and both volumetric bone density and bone size at weight-bearing sites in girls (164). See section 3.3.3.4 on page 92 for a further discussion of the effects of physical activity.

3.2.1.2. Parental size

Non-modifiable determinants of bone mass in childhood include parental size as an adult and parental birth weight.

Parental size as an adult

Studies were found that investigated the association between parental size as an adult and bone mass in their offspring. Height of the mother was positively associated with estimated volumetric bone density of the spine in offspring, independent of gestational age (167). Paternal bone area has also been shown by the same group to predict neonatal bone area, independently of mothers body build (168).

Parental birth weight

The same study above that looked at parental height also looked at parental birth weight (167) and found no associations between maternal birth weight and estimated

volumetric bone density of either the spine or total body of offspring. Unadjusted total body and spine BMC and BMD were positively associated with maternal birth weight, but BMAD was not, indicating the influence of maternal birth weight on body size of offspring.

3.2.1.3. Pubertal stage

Timing of puberty in boys and girls

During puberty the growth spurt occurs at different stages in boys and girls. For girls, the growth spurt occurs at the start of breast and pubic hair development, with menarche (start of menstruation) occurring towards the end of puberty (see Figure 11, page 68). For boys, puberty is already well established (testicular volume 10-12mls and pubic hair present) by the time the growth spurt occurs (see Figure 12, page 69). This has important implications for investigating any potential relationships between bone density, fractures and pubertal stage as assessed by the Tanner Staging as body size must be adjusted for.

Physical changes of puberty: Tanner staging

The most common and validated method of assessing an individual child's current pubertal development is the Tanner staging of puberty first published by James Mourilyan Tanner, a British Paediatrician, in 1962 (169). Tanner stage is assigned by comparison of the child with published pictures of the five stages of breast development for girls or penis and testis development for boys, and the five stages of pubic hair development for both genders (see Table 7, page 70).

Originally, these stages were designed to be assigned by a paediatrician after direct observation of the child. However, with the increasing use of postal questionnaires, self-report by the child after being given a copy of the diagrams is being used instead of direct observation. Two studies were found that compared self-report of Tanner staging with direct observation by a paediatrician (170,171). The first study (170) used 43 females and 23 males and reported excellent agreement (kappa coefficients of 0.81 for girls breast stage, 0.91 for girls pubic hair development and 0.88 for males combined pubic hair and genital stage). The second study (171) looked at 151 girls and found a correlation between self-reports and paediatrician direct observations of 0.82.

Effect of sex steroids on bone and mineral metabolism

In both males and females, cross-sectional analyses have reported that oestrogens (specifically estradiol) concentrations predict bone mass more accurately than testosterone concentrations. Oestrogens repress osteoclastogenesis (172), promote epiphyseal maturation (173), stimulate endosteal and trabecular bone formation (174), augment mineralisation and increase tensile bone strength (175). The direct effects of testosterone on bone are not fully understood but include increased insulin-like growth factor one (IGF-I) synthesis in bone cells (176), increased skeletal retention of calcium and magnesium (177), increased biochemical markers of osteoblastic activity such as osteocalcin (178), and epiphyseal growth-plate maturation (179,180).

Figure 11: Pubertal development in girls. *T2* to *T5* indicate approximate timing of Tanner stages of breast and pubic hair development.

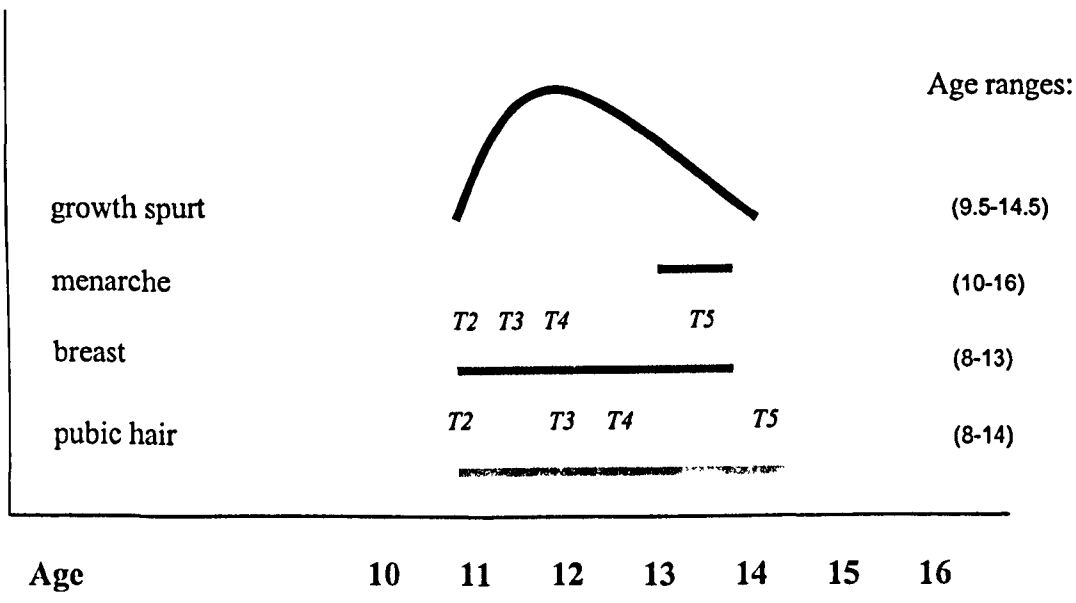
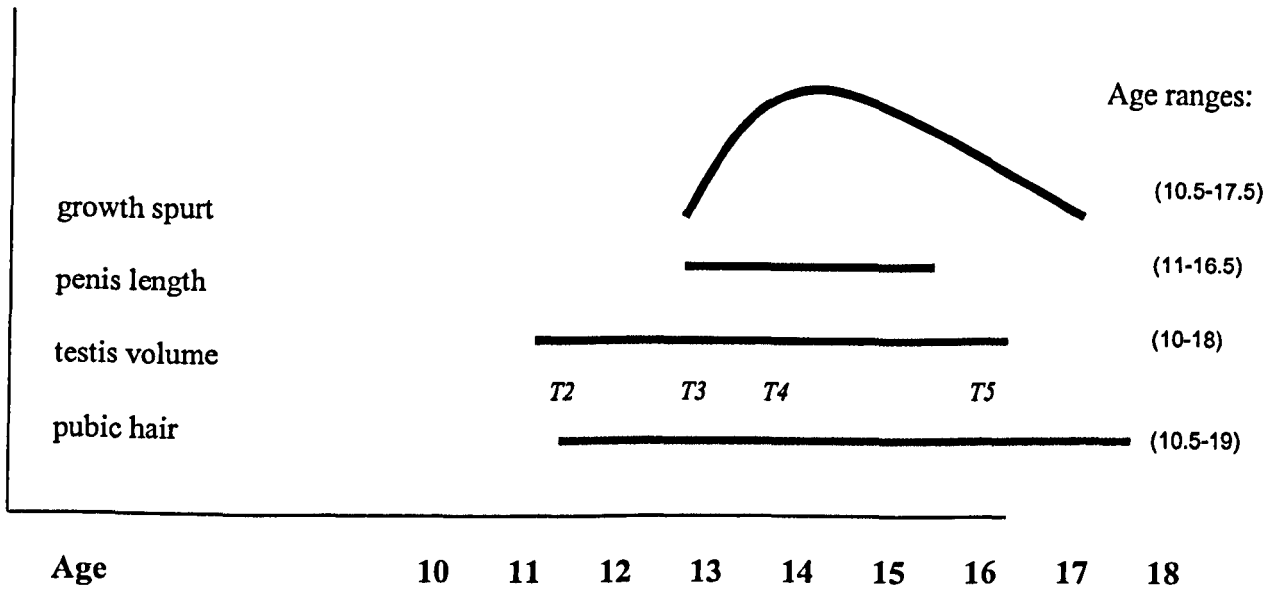


Figure 12: Pubertal development in boys. *T2* to *T5* indicate approximate timing of Tanner stages of pubic hair development.



Clinically, in young men serum free estradiol has been reported to be a negative predictor of cortical bone CSA, periosteal circumference and endosteal circumference at the tibia and radius, but a positive predictor of measured volumetric bone density by pQCT (181). In early pubertal girls (182) estradiol was a negative predictor of marrow cavity proportion but a positive predictor of both cortical thickness and measured volumetric bone density of the tibia by pQCT. Conversely, in the same study on young men (181), free testosterone was an independent positive predictor of cortical CSA, periosteal circumference and endosteal circumference at the tibia and radius, but was not associated with volumetric bone density. In the study on early pubertal girls (182) testosterone had no detectable effect on bone size or volumetric density.

Table 7: Description of the Tanner stages of puberty

Tanner Stage of male genitals:	
Stage 1	Testes small in size with childlike penis
Stage 2	Testes reddened, thinner and larger (1.6-6ml) with childlike penis
Stage 3	Testes larger (6ml-12ml) and scrotum enlarging. Increase in penile length
Stage 4	Testes larger (12ml-20ml) with greater enlargement and darkening of the scrotum. Increase in length and circumference of penis
Stage 5	Testes over 20ml with adult scrotum and penis
Tanner stage of female breast:	
Stage 1	No breast tissue with flat areola
Stage 2	Breast budding with widening of the areola
Stage 3	Larger and more elevated breast extending beyond the areola
Stage 4	Larger and even more elevated breast. Areola and nipple projecting from the breast contours
Stage 5	Adult size with nipple projecting above areola
Tanner stage of male and female pubic hair:	
Stage 1	None
Stage 2	Small amount of long hair at base of male scrotum or female labia majora
Stage 3	Moderate amount of curly and coarser hair extending outwards
Stage 4	Resembles adult hair but does not extend to inner surface of thigh
Stage 5	Adult type and quantity extending to the medial thigh surface

Association between pubertal stage and volumetric bone density

Total body estimated volumetric bone density increases from prepuberty to postpuberty by approximately 43% in boys and by 66% in girls (127). The timing of maximal accumulation of volumetric bone density is not completely clear, but probably occurs in the later stages of puberty (127,183,184), after peak height velocity has occurred (185). In girls, the incremental rate of bone mass peaks at menarche (183,186). There is evidence of a transient decline in volumetric bone density at the radius in early puberty, due to an acceleration in the rate of growth of bone size before the peak growth of mineralisation (187). There is also some evidence that volumetric bone density of the

hip (127,188) and femur (189) does not change during puberty, possibly because these areas reach adult levels before puberty (188), but not all studies agree (190).

Association between pubertal stage and bone size

A summary of the studies found investigating the association between puberty and change in bone size is shown in Table 8 on page 72. These all confirm that bone size increases during puberty. The correlation between growth of specific bones and peak height velocity appears to be different for growth of bones in length and growth of bones widthways. For example, peak growth in length of the second metacarpal bone coincides with peak height velocity (191) but peak growth of metacarpal cortical thickness occurs later. Growth in length of limbs occurs before growth in height of vertebral bodies (188).

Growth widthways of long bones can be measured by increase in CSA and can occur by periosteal expansion (apposition), with or without endosteal resorption. The ratio of increase in periosteal expansion to endosteal resorption determines cortical thickness (see Figure 2 on page 25). Two studies report on the increase in CSA of long bones during puberty, but show different mechanisms. One (192) reports periosteal apposition and endosteal resoprtion occurring in both sexes during puberty, with no evidence of endosteal apposition in girls. Boys showed a greater magnitude of change at both the periosteal and endosteal surfaces resulting in a greater increase in bone size during puberty. The other study (187) just investigated girls and reports that bone formation occurred at the periosteal surface before menarche, and both periosteal and endocortical apposition occurred after menarche. During puberty the ratio of cortical-to-total CSA of the tibial shaft increased, and that of marrow-to-total CSA decreased.

Association between pubertal stage and biochemical markers of bone turnover

During puberty, bone marker levels are four to five times higher than adult levels reflecting high bone turnover (183). The highest concentrations of markers of both bone turnover and formation are reported in the early stages of puberty (189), mid puberty (147), or different stages depending on gender (147) indicating that pubertal stage is a main determinant of bone marker levels.

Table 8: Summary of the association between puberty and change in bone size

Author, year of publication, study design	No in study	Measure of pubertal status	Measure of bone size used	Result
Mora et al, 1999, USA. Cross-sectional. (189)	269	Tanner stage - 62 in stage 1, 34 in stage 2, 50 in stage 3, 81 in stage 4, 42 in stage 5	CSA L vertebrae and CSA and cortical bone area of midshaft femur by qCT	CSA L vertebrae and CSA and cortical bone area of midshaft femur all increased with pubertal stage.
Hogler et al, 2003, Australia. Cross-sectional. (193)	145	Tanner stage (unknown how many in each stage)	bone geometry of mid-femur by MRI: CSA, total bone area, cortical bone area and medullary bone area	Total bone area, CSA and medullary bone area all increased with puberty. Cortical thickness also increased, as the increase in cortical bone area was slightly more than the increase in medullary bone area showing that both periosteal and endocortical expansion occur at the mid-femur.
Blimkie et al, 1993, Belgium. Prospective cohort. (191)	278 boys	Peak height velocity	metacarpal length and cortical thickness by radiogrammetry	Ages at peak velocity of height and metacarpal length were similar. Age at peak velocity of metacarpal cortical thickness later than peak height velocity.
Kontulainen et al, 2005, Canada. Prospective cohort. (192)	128	Tanner stage and age at menstruation, then divided into 3 groups according to characteristics by end of study	total CSA, cortical bone CSA, ratio of total to cortical CSA, marrow cavity area of tibial midshaft by pQCT	Both genders showed an increase in cortical bone CSA during puberty with both periosteal apposition and endosteal resoprtion occurring, but greater periosteal apposition than endosteal resoprtion. Boys showed a greater magnitude of change at both the periosteal and endosteal surfaces resulting in a greater increase in bone size during puberty. No evidence of endosteal apposition in girls.
Sundberg et al, 2003, Sweden. Prospective cohort. (188)	86	Tanner stage	Total length of spine, upper limb and lower limb, and width of femoral neck by DXA	Growth in length of limbs preceded growth in length of spine at every Tanner stage.
Wang et al, 2005, Finland. Prospective cohort. (187)	258 girls	date of menarche	total, cortical and marrow cavity CSA and cortical thickness of radius and tibia, by pQCT	At radius, growth rate of CSA peaked before growth rate of BMC so total measured volumetric bone density of radius declined initially, before increasing towards end of puberty. At tibial shaft timing of CSA and BMC growth rates were similar. Cortical thickness increased linearly (bone formation at periosteal surface before menarche, both periosteal and endocortical apposition after menarche). During puberty ratio of cortical-to-total CSA of the tibial shaft increased, and that of marrow-to-total CSA decreased.

Abbreviations: BMC bone mineral content; CSA cross-sectional area; DXA dual energy X-ray absorptiometry; L lumbar; pQCT peripheral quantitative computed tomography; qCT quantitative computed tomography; USA United States of America

3.2.1.4. Other hormonal/endocrine influences on bone mass in childhood

Parathyroid hormone (PTH) is consistently reported as being inversely related to volumetric bone density in children (194-196), confirmed by a positive association with the bone turnover marker osteocalcin (196). One study suggests PTH is also positively associated with bone area measured by DXA and CSA of the radius and tibia by pQCT (195). Its mechanism of action may include modification of the set-point of the growth hormone (GH) / IGF-I axis, which has been suggested to play a role in the programming (see Chapter 2, page 44) of bone development by early life factors (197).

GH, IGF-I, IGF-II and IGF-binding proteins control growth, remodelling and mineralisation of the skeleton in part by direct actions on bone (175). Specifically, GH stimulates skeletal IGF-I synthesis (198), proliferation of prechondrocytes (199), hypertrophy of osteoblasts (200), bone remodelling and net mineralisation after a time-lag of 1-2 years (201). Clinically, IGF-I measured in cord blood is closely related to the size of the neonatal skeleton (202), and when measured in adolescents correlates with tibial cross-sectional area and cortical bone area (203). These two studies also suggest that IGF-I does not appear to be related to bone density, but a further study on 11-13 years old girls produced contradictory findings (204).

Leptin is a protein produced by adipocytes and the placenta and participates in the regulation of food intake and energy expenditure (205). It is also necessary for pubertal development in mice, and is increased in prepubertal children independent of adiposity, suggesting a role in childhood growth and development (206). The literature on the association between leptin and volumetric bone density in children is contradictory: two studies suggest a positive association (202,207), and four report no association (208-211). Similar contradictory results have been reported in studies that have investigated the association between leptin levels and bone size in children with two reporting a positive association (207,212) and one a negative association (211).

There appears to be a multihormonal control of bone growth and maturation with the main hormones already discussed. Other hormones such as cortisol and thyroxine act on bone via local effector molecules such as cytokines, prostaglandins and osteoprotegerin (175).

3.2.1.5. Summary of the non-modifiable determinants of bone mass in children

This section has presented the literature on the non-modifiable determinants of volumetric bone density and bone size in childhood. Bone mass in childhood increases with age, and although boys have bigger bones there appears to be little gender difference in volumetric bone density before puberty. Puberty has a fundamental role in the acquisition of bone mass, mainly acting via the multi-hormonal pathways discussed. The genetics of childhood bone mass is an emerging area of study, and may help increase our knowledge of the biology of bone formation and breakdown. However, in general, because of contradictory results or results from only single studies, no conclusions can be drawn about the genetic determinants of childhood bone mass. Also, the functional significance of most of the polymorphisms studied are unknown. Parental size, both at birth and as an adult has a positive influence on childhood bone mass. PTH is inversely related to volumetric bone density, while IGF-I is positively associated with bone size. The next section discusses the early life determinants of bone mass in childhood, both intra-uterine factors and those acting during early post-natal life.

3.2.2. Early life determinants of bone mass in childhood

The previous section presented the literature on the non-modifiable determinants of volumetric bone density and bone size in childhood. This section focuses on early life determinants of childhood bone mass and is divided into two sections: factors acting during intra-uterine life; and those acting during early post-natal life.

3.2.2.1. Factors acting during intra-uterine life

Maternal diet during pregnancy

There is evidence from animal studies that protein restriction in maternal rats is associated with reduced bone area and BMC in offspring. The offspring also show a delay in the formation of osteogenic precursors within the bone marrow compartment and altered growth plate morphology (213,214). A few observational studies have looked at diet during pregnancy in humans, and childhood bone mass, but the most recent, and largest study (215) suggests that in populations with adequate nutrition, maternal diet during pregnancy makes a relatively small contribution (R^2 value of approximately 0.5%) towards bone mass in childhood. In univariable analyses, many nutrients are reported to be associated with offspring bone mass. However, as there is strong co-linearity between nutrients, in multivariable analyses the evidence is contradictory depending on adjustments. Many nutrients appear to influence bone mass indirectly through actions on height or weight. More evidence is needed before firm conclusions can be drawn.

Calcium supplements given during pregnancy appear to increase offspring volumetric bone density only if the mothers had poor dietary intake of calcium (216,217). There is no evidence that maternal vitamin D intake or levels is associated with offspring estimated volumetric bone density (215,218). A summary of the available evidence on the association between maternal nutrition and volumetric bone density of offspring is shown in a tabular form on page 76. Two studies were found (218,219) that investigated the effects of maternal nutrition during pregnancy on bone size of offspring. The study by Javaid et al (218) report a positive association between vitamin D intake and

Table 9: Table showing the associations between maternal intake of various nutrients during pregnancy, and effect on volumetric bone density of offspring.

Author, year of publication	Age of participants	No in study	Nutritional status of population	Maternal dietary constituents studied	Measure of volumetric bone density used	Result
Observational studies						
Javaid et al, 2006, UK (218)	9 years old	160	good	vitamin D	size-adjusted lumbar spine BMD by DXA	No association between vitamin D intake and estimated volumetric bone density of spine at aged 9 years. Vitamin D status associated with greater bone area in offspring at aged 9 years
Tobais et al, 2005, UK (215)	9.9 years	4451	good	calcium, vitamin D, sodium, magnesium, potassium, phosphate, zinc, folate, vitamin C, vitamin E, retinol, riboflavin, carotene, thiamin, niacin, fat, fibre, carbohydrate, starch, sugar, protein	size-adjusted total body and spine BMC by DXA	Only independent association found was between maternal folate intake and estimated volumetric bone density of spine, but not total body, at aged 9.9 years
Weiler et al, 2005, Canada (220)	neonates	30	good	long-chain polyunsaturated fatty acids	size-adjusted whole body BMC by DXA	No association between maternal long-chain polyunsaturated fatty acids and estimated volumetric bone density
Godfrey et al, 2001, UK (167)	neonates	145	good	calcium, green vegetables	size-adjusted spine BMD by DXA	No association with maternal calcium intake. Negative association with maternal green vegetable consumption (P=0.002)
Jones et al, 2000, Southern Tasmania (221)	8 years	173	good	calcium, magnesium, phosphorus, potassium, protein, fat	size-adjusted femoral neck, L spine and total body BMC by DXA	Maternal phosphorus and fat intake were independently and positively associated with estimated volumetric density of L spine, but not femoral neck or total body

Table 9, continued

Author, year of publication	Age of participants	No in study	Nutritional status of population	Maternal dietary constituents studied	Measure of volumetric bone density used	Result
Interventional studies						
Koo et al, 1999, USA (216)	neonates	256	mixed	calcium supplementation of 2g per day compared with placebo	size-adjusted total body and L spine BMC by DXA	Positive effect of supplements only if mothers had poor dietary intake of calcium
Raman et al, 1978, India (217)	neonates	87	poor	calcium supplementation of 300 and 600mg per day compared with placebo	size-adjusted bone density of ulna, radius, tibia and fibula by radiogrammetry	Positive association between calcium supplements and estimated volumetric density of all bones measured

Abbreviations: BMC bone mineral content; BMD bone mineral density; DXA dual energy X-ray absorptiometry; L lumbar; mg milligram; UK United Kingdom; USA United States of America

whole body bone area, and the study by Chang et al (219) report a positive association between maternal calcium intake and foetal femur length in-utero.

Maternal smoking during pregnancy

Five studies have been found that investigated the association between maternal smoking during pregnancy and offspring bone mass (87,149,167,222,223). Two studies found a negative association between maternal smoking and estimated volumetric bone density (167,223), and the others showed no association. In the study published in 1999 based on DXA scans from 330 8-year old children from Tasmania (223), mothers who smoked also had lower placental weights (-56g, 95%CI -95 to -17), and further adjustment for placental weight led to nonsignificant results for smoking with bone growth and bone parameters, suggesting that these associations may be mediated through placental size and function. However, the study (167) from Southampton in the UK published in 2001, found no association with smoking at conception or during pregnancy and placental weight.

Other maternal factors during pregnancy and offspring bone mass

Other maternal factors that have been shown to be associated with lower bone mass of offspring include higher physical activity levels of the mother in late pregnancy (167), maternal diabetes during pregnancy (224,225) and use of medications such as magnesium sulphate (226,227) for delaying delivery (tocolysis). Season of birth also appears to be associated with bone mass of offspring (228,229) perhaps due to reduced maternal exposure to sunshine in early pregnancy causing vitamin D deficiency and affecting foetal bone development, such that summer born infants (who would have been in their first trimester during winter) have a lower bone mass than winter born infants.

Gestational age and birth weight or birth length

Gestational age and birth weight reflect the intra-uterine environment and are inter-related as premature babies have a lower birth weight than babies born at term. I have reviewed the literature on the association between size-adjusted gestational age and bone mass, and have taken this to represent the effects of maturity or prematurity on

bone mass, and consequently have focused on average-weight for gestational age (AGA) infants. To try and distinguish between prematurity and low birth weight as a result of intra-uterine growth retardation (IUGR) I have reviewed the literature on birth weight adjusted for gestational age, and also focussed on small for gestational age (SGA) infants.

Gestational age

It is a consistent finding that gestational age is positively associated with neonatal estimated volumetric bone density (230-235). However, it is less certain whether being premature results in low volumetric bone density in the longer term. Three studies were found that looked at this (231,236,237). Two report that those children born prematurely did not have as high estimated volumetric bone density as those born at term even when aged 6 months (231) or 7 to 9 years (236). Conversely, a further study (237) found that by 17-years of age, although the former preterm young adults aged 17 years were shorter (164.8cm versus 172.1cm, $P<0.05$) and had a lower whole body BMC (2087g versus 2326g, $P<0.05$) than those born at term, BMC was appropriate for size i.e. after adjusting for body size, whole body BMC was no longer different in the two groups (12.6g/cm versus 13.4g/cm, $P>0.05$).

Birth weight and birth length adjusted for gestational age

It is a consistent finding that after adjusting for gestational age, birth weight (230,231,238,239) and birth length (230,231,239) are positively associated with bone mass. Birth weight and birth length are reported to account for up to 68-71% of the variability of neonatal bone mass (239).

Small for gestational age

To assess the impact of IUGR on bone mass a few studies have been found that assessed bone mass in babies small for gestational age (SGA) compared to those appropriate for gestational age (AGA), but the results are contradictory. One study was found that reported no difference in estimated volumetric bone density in 15 preterm SGA and 43 preterm AGA infants from Finland (240). Three studies have been found that looked at bone turnover markers in SGA and AGA neonates and the results are contradictory and based on small numbers (241-243).

Summary of factors acting during intra-uterine life to influence childhood bone mass

Maternal diet during pregnancy makes a relatively small contribution (R^2 value of approximately 0.5%) towards bone mass in childhood, with calcium intake appearing to be important for volumetric bone density. Maternal smoking during pregnancy may have a detrimental effect on childhood bone mass. Gestational age and birth weight or birth length are positively associated with neonatal and infant estimated volumetric bone density, but it is uncertain whether long-term effects of these determinants are seen on bone mass in later childhood or young adulthood. Size at birth, particularly birth weight and birth length, account for around 70% of the variability in neonatal bone mass. It is not possible to draw any conclusions on the effects of IUGR on childhood bone mass due to a small number of contradictory reports.

3.2.2.2. Factors acting during early post-natal life

Breast-feeding versus formula feeding

Many studies have been carried out looking at the association between early life feeding regimes and bone mass in the infants (Table 10 and Table 11, starting on page 81). Observational studies are contradictory with one study suggesting breast-feeding is associated with a lower estimated volumetric bone density than bottle feeding (244), one suggesting breast feeding is associated with a higher bone mass (245), and four showing no association (246-249). Interventional studies, mostly randomised controlled trials (RCTs), are also contradictory. In these studies, the infants whose mothers elected not to breastfeed were randomised to different types of formulae, and then compared with a concurrently recruited human milk-fed group. Two studies showing lower bone mass in infants who are breast fed (250,251), and three showing no association between various feeding methods and bone mass (252-254). The most recent RCTs with the largest numbers of infants participating are the studies that have shown no association, but these studies have been carried out in well-nourished populations. The RCT by Specker et al (250) showed lower size-adjusted total body BMC measured at 6 months in infants breast-fed compared to those fed a low- or moderate-mineral containing formula, but by 12 months there was no difference in bone mass between any of the groups. It also does not seem to matter if the babies were premature or healthy term babies.

Table 10: Literature review of breast feeding versus formula feeding and impact on childhood bone density: Observational studies

Author, year of publication	Type of study	Population	N	Age at measurement of bone mass	Measure of volumetric bone density	Result
de Schepper et al, 2005. Belgium (246)	Birth cohort	premature babies	34	discharge from hospital (median of 40 days)	total body BMC as a percentage of body mass by DXA	No association between diet (fortified breast milk or preterm formula) and total body estimated volumetric bone density. Actual values not available from abstract
de Curtis et al, 2001. Belgium (247)	Birth cohort	healthy term	27	on enrolment (3 days) and 2 months	size-adjusted total body BMC by DXA	No association between diet (breast fed or formula fed) and estimated total body volumetric bone density: 134 ± 36 in breast fed group versus 136 ± 54 in formula fed group
Jones et al, 2000. Tasmania (245)	Birth cohort	children at high risk of SIDS	326	8 years	size-adjusted femoral neck, L spine and total body BMD by DXA	Association only seen in children born at term, not in premature children: breast feeding for 3 months or longer was associated with increased estimated volumetric bone density at all sites: e.g lumbar spine +0.25SD, P=0.03
Butte et al, 2000. USA (244)	Birth cohort	healthy term	76	0.5, 12 and 24 months	size-adjusted total body BMC by DXA	Breast fed infants had a lower estimated volumetric density at 12 months compared to those fed formula. By 24 months no difference was observed. Actual values not given in paper
Fewtrell et al, 1999. UK (248)	Long-term follow-up of two RCTs	premature babies	183 and 61	8 to 12 years	size-adjusted total body, L spine, femoral neck and radius BMC by DXA	No association between diet for first 30 days of life (banked human milk, preterm formula or term formula) and estimated volumetric bone density of any site: e.g for L spine 24.93 ± 5.91 in banked human milk, 23.35 ± 5.49 in pre-term formula and 26.66 ± 7.93 in term formula
Park et al, 1998. Korea (249)	Cross-sectional	healthy term aged 2 to 5 mo	35	2 to 5 months	size-adjusted L spine BMC by DXA	No association between current feeding practices (breast feeding or formula) and estimated volumetric density of L spine: 0.62 ± 0.2 versus 0.65 ± 0.2 for formula

Abbreviations: BMC bone mineral content; BMD bone mineral density; DXA dual energy X-ray absorptiometry; L lumbar; mo months; RCT randomised controlled trial; SD standard deviation; UK United Kingdom; USA United States of America

Table 11: Literature review of breast feeding versus formula and impact on childhood bone density: Interventional studies

Author, year of publication	Type of study	Population	N	Age at measurement of bone mass	Measure of volumetric bone density	Result
Faerk et al, 2000. Denmark (252)	RCT	premature babies	127	at term	size-adjusted total body BMC by DXA	No association between diet for first 38 ± 14 days of life (phosphate supplementation of human milk, fortified supplementation of human milk with protein, calcium and phosphorus, or preterm formula) and estimated volumetric bone density: 45.7 ± 8.2 in phosphate group, 46.6 ± 9.9 in fortifier group and 46.6 ± 10.6 in pre-term formula group
Specker et al, 1997. USA (250)	RCT	healthy term	101	1, 3, 6, 9 and 12 months	size-adjusted total body BMC by DXA	Lower estimated volumetric density in infants breast fed for 6 months compared to those fed a low or moderate-mineral containing formula e.g 158.7 ± 2.4 for breast fed, 157.6 ± 2.5 for low-mineral and 168.7 ± 2.5 for moderate mineral groups. However, at 12 months there was no difference between the 3 groups: 233 ± 5 for breast fed, 237 ± 3 for low mineral and 236 ± 3 for moderate
Mimouni et al, 1993. USA (253)	RCT	healthy term	72	2, 4, 6 and 12 months	width-adjusted BMC distal radius by single-beam photon absorptiometry	No association between diet for the first 4 months of life (human milk, cow milk-based formula or soy-based formula) and estimated volumetric density at any time point: e.g at 12 months 202 ± 9 for breast fed, 208 ± 7 for soy-based formula and 229 ± 11 for cow-based formula
Horsmann et al, 1989. UK (251)	RCT	premature babies	36	at term	size-adjusted BMC of forearm by single-beam photon absorptiometry	Higher bone mineral accretion in group receiving supplemented formula for first 15 weeks of life, compared to those fed breast milk or non-supplemented formula, P<0.02. Actual values not given in paper.
Hillman, 1988. USA (254)	3 arms - unknown if randomised	healthy term	31	at birth, 2, 4, 6, 9 and 12 months	mid-humerus size-adjusted BMC by single-beam photon absorptiometry	No difference was seen in estimated volumetric density between the three groups (human milk, cow milk-based formula or soy-protein based formula. Actual values not given in paper

Abbreviations: BMC bone mineral content; BMD bone mineral density; DXA dual energy X-ray absorptiometry; RCT randomised controlled trial; UK United Kingdom; USA United States of America

Vitamin D supplementation of early life feeding regimes

Vitamin D is involved in the regulation of calcium metabolism and three observational studies (249,255,256) and two RCTs (257,258) have been found that investigated the association between early life vitamin D status and bone mass children, and the results are contradictory. Two observational studies report higher estimated volumetric bone density of the femoral neck in vitamin D supplemented children (255,256), and one found no association (249). The results of the RCTs are similar as both showed an increase in infant estimated volumetric bone density with early life vitamin D supplementation, e.g. at 3 months old size-adjusted BMC of the distal radius was 36% higher in the 1000IU/day vitamin D supplemented group (258). However, one study repeated the bone mass measurements at aged 9- to 11-years and found no difference in bone mass at this age (258).

Summary of factors acting during early post-natal life to influence childhood bone mass

Also, the literature is inconsistent as to whether breast-feeding or formula feeding is best for bone mass. Evidence for an effect of vitamin D supplementation in infancy on bone mass is also contradictory.

3.2.2.3. Summary of the early life determinants of bone mass in childhood

This section reviewed the literature on the early life determinants of childhood bone mass, both intra-uterine and early post-natal factors. Maternal diet during pregnancy makes a relatively small contribution (R^2 value of approximately 0.5%) towards bone mass in childhood, with calcium intake appearing to be important for volumetric bone density. Gestational age is positively associated with volumetric bone density of neonates. Size at birth, particularly birth weight and birth length, account for around 70% of the variability in neonatal bone mass. The next section discusses the potentially modifiable determinants of bone mass in childhood.

3.2.3. Potentially modifiable determinants of bone mass in childhood

The previous two sections have discussed the non-modifiable and early life determinants of bone mass in childhood. This section focuses on the potentially modifiable determinants and is divided into five sections: diet and nutrition; socio-economic status; body composition; physical activity; and medications.

3.2.3.1. Diet and nutrition

Many papers have been published that look at the association between dietary intake of calcium and bone mass in childhood and the literature is reviewed below with a summary table starting on page 85.

A range of observational studies, both cross-sectional and prospective cohort studies have reported a positive association between dietary vitamin D intake and volumetric bone density in childhood or adolescence, either estimated by DXA (255,259,260) or measured directly by pQCT (194). One study reported no association (195).

Two studies have reported a negative association between carbonated beverage intake (261,262) and estimated volumetric bone density in childhood. It is thought this negative association is due to milk displacement from the diet rather than direct effects on bone from the components of carbonated drinks such as phosphorus (263), perhaps providing more evidence on the importance of calcium intake for bone health in childhood. Small studies, often as part of a subgroup analysis, have investigated the associations between vegetable intake and estimated volumetric bone density in childhood with two suggesting a positive association (264,265) and one a negative association (266).

Calcium intake

In general, observational studies of the association between calcium intake and childhood bone mass are contradictory (267-269). A summary of the evidence from RCTs is shown in Table 12 on page 85. Seven RCTs showed increases in estimated volumetric bone density in the calcium supplemented groups (270-276) but one showed no association (277). Five reported an increase in bone size (276-280) but six showed no association between calcium supplementation and change in bone size

Table 12: Summary of the literature review on the effects of dietary calcium on bone mass in children.

Author, year of publication	Number of participants (age)	Study design	Measurement of volumetric bone density used	Measurement of bone size used	Other measures of bone status	Main result
Matkovic et al. USA, 1990 (279)	28 girls (14 years)	Supplemented group (n=20) received either milk or calcium carbonate tablets (250mg calcium each). Follow-up for 24 months		cortical area by radoigrammetry		Increased bone size in combined supplemented groups compared to placebo ($P<0.05$). Actual values not given in paper
Johnston et al, USA, 1992 (281)	90 (10 ± 2 years)	supplemented group (one of a pair of twins) received 1000mg calcium citrate malleate per day). Follow-up for 3 years		area and width of radius, L spine, femoral neck by DXA		No change in bone size between those given calcium daily and placebo
Lee et al, Hong Kong, 1994 (270)	162 (7 years)	Supplemented with 300mg calcium carbonate or placebo. Follow-up for 18 months	size adjusted BMC of radius by DXA	width of radius by DXA		Greater gains in estimated volumetric density of radius in those given calcium (9.45% vs 6.31%, $P=0.0008$). No change in bone width.
Lloyd et al, USA, 1996	112 girls (12 ± 0.5 years)	Supplemented with 500mg calcium citrate malate or placebo. Follow-up for 24 months	size-adjusted BMC and BMD of total body and L spine by DXA	area of total body and L spine		No difference in bone size between supplemented and placebo group. Greater estimated volumetric density in supplemented group (12.2% increase vs 10.1%, $P=0.005$)
Cadogan et al, UK, 1997	82 girls (12.2 ± 0.3 years)	Supplemented with one pint of milk per day or nothing. Follow-up for 18 months	size-adjusted BMD and BMC of total body by DXA		Markers of bone formation: osteocalcin, BAP Markers of bone resorption: U-NTX, DPD	Increased estimated volumetric density in supplemented group (9.6% vs 8.5%, $P=0.017$) but no difference in bone turnover markers.

Table 12, continued

Author, year of publication	Number of participants (age)	Study design	Measurement of volumetric bone density used	Measurement of bone size used	Other measures of bone status	Main result
Bonjour et al, Switzerland, 1997 (282)	149 (8 years)	Given a food product (cake, yoghurt etc) supplemented with 850mg calcium or placebo. Follow-up for 12 months		Change in L spine bone area, width and height by DXA. Change in femoral shaft bone area and width		Increased height of L vertebrae and femoral neck bone size in those given two food products containing calcium. Greater increases seen in those with a spontaneous calcium intake below the median.
Dibba et al, Gambia, 2002 (271)	106 (8.3-11.9 years)	Supplemented with either 1000mg calcium carbonate or placebo. Follow-up for 12 months	size-adjusted BMC of distal radius by DXA	width of radius by DXA		Estimated volumetric density was higher in supplemented group (5.0 ± 1.1%, P<0.0001). No difference in bone size.
Cameron et al, 2004, Australia	102 girls (10.3 ± 1.5 years)	One pair of each twin received 1200mg calcium carbonate per day vs placebo. Follow-up for 24 months	size-adjusted BMD of total body, hip and L spine			Increased estimated volumetric density at hip and L spine in supplemented group. eg for hip 2.4% increase vs 0%, P<0.001
Matkovik et al, 2005, USA	354 girls (11-12 years)	Supplemented with 1000mg calcium citrate malate or placebo. Follow-up for 4 years		area of total body and radius by DXA. Metacarpal total and cortical area by radiogrammetry	Markers of bone formation: osteocalcin, BAP Markers of bone resorption: U-NTX	Increased ratio of cortical to total metacarpal area in supplemented group (0.427 ± 0.042 vs 0.414 ± 0.047, P<0.01). No effect on area measured by DXA. No effect on bone turnover markers
Chevalley et al, 2005, Switzerland	235 boys (7.4 ± 0.4 years)	Supplemented with 850mg calcium or placebo. Follow-up for 12 months	size-adjusted BMD of radius, hip and L spine by DXA	area of radius, hip and L spine by DXA		Increased estimated volumetric density of radius and hip in supplemented group, but no effect at L spine. No effect on bone size.

Table 12, continued

Author, year of publication	Number of participants (age)	Study design	Measurement of volumetric bone density used	Measurement of bone size used	Other measures of bone status	Main result
Prentice et al, 2005, UK	143 boys (16-18 years)	Supplemented with 1000mg calcium carbonate or placebo. Follow-up for 13 months	size-adjusted BMC total body, L spine and hip by DXA	area of hip and radius by DXA		Increased estimated volumetric density of hip (1.09% ± 0.54, P<0.05) but no association with other estimates of volumetric density. Increased area of L spine in supplemented group (1.52% ± 0.5, P<0.01)
Cheng et al, 2005, Finland	195 girls (10-12 years)	Supplemented with 1000mg calcium, 1000mg calcium plus vitamin D3 (200IU), cheese containing 1000mg calcium or placebo. Follow-up for 24 months	volumetric density of radius and tibia by CT	area of total body, femoral neck and L spine by DXA. CSA of radius and tibia, cortical bone thickness of tibia by CT		No effect of measured bone density of radius or tibia. No effect on area of total body, femoral neck and L spine measured by DXA. No effect on CSA of radius and tibia, but increased cortical bone thickness of tibia in the cheese supplemented groups than any of the other groups.
Zhu et al, 2005, China	757 girls (10-12 years)	Randomly assigned to calcium-fortified milk, calcium and vitamin D-fortified milk or placebo. Follow-up for 24 months		Periosteal diameter, medullary diameter, cortical thickness and length of second metacarpal by radoigrammetry	Markers of bone formation: osteocalcin, BAP Markers of bone resorption: DPD	Increased cortical thickness and periosteal diameter, but smaller medullary diameter in supplemented groups. eg 5.7% gain in cortical thickness, P<0.05. Lower BAP in group who received calcium and vitamin D-fortified milk

Abbreviations: BAP bone specific alkaline phosphatase; BMC bone mineral content; BMD bone mineral density; CT computed tomography; DPD deoxypyridinoline; DXA dual energy X-ray absorptiometry; RCT randomised controlled trial; U-NTX urinary N-terminal cross-linking telopeptide of type I collagen; UK United Kingdom; USA United States of America

(270,272,273,277,281,283). Two show no association with changes in bone turnover markers (275,278) but one reports lower serum BAP at 12 months in the control group indicating reduced bone formation (280). In conclusion, general agreement of six review papers (284-289) is that there is a modest positive association between calcium intake and volumetric bone density in children and adolescents.

The difficulties with investigations into associations between calcium intake and bone mass in childhood are numerous. The associations found may be confounded by total energy intake (289,290), protein intake (289,291), physical activity (292) or gender (293). There are also suggestions that the timing of calcium intake in relation to pubertal status is important: there may be a greater effect of calcium in pre- or post-pubertal children rather than those in mid-puberty (272,289,294). Another unknown is whether the positive associations between calcium supplementation and bone mass seen in the RCTs results in a long-lasting effect, or a transitory effect (289). Three long-term follow-up studies report that several years after stopping calcium supplementation, an effect on bone density is not maintained (274,295,296). However, three different follow-up studies suggest that the effect is long-lasting (278,297,298).

3.2.3.2. Socio-economic status

No studies have been found that examine the relationship between social status and bone mass acquisition in childhood. However, there is reason to believe that social status may influence bone mass acquisition in childhood since nutrition and physical activity both show social gradients (299,300). Socio-economic factors have also been found to be related to growth in childhood as, for example, Rona reported a difference in height of approximately 3cm between the two extremes of social class at age seven (301). The possibility that social factors play an important role in determining skeletal growth and peak bone mass acquisition is consistent with several previous studies in which a positive association has been observed between socio-economic status and bone mass in adult populations (302-305). However, other studies on adults have found either no association, or a negative relationship (306-308).

3.2.3.3. Body composition

For this section I have divided body composition into lean mass (muscle), fat mass and height. I have not reviewed the literature in detail for the association between body weight or body mass index (BMI) and bone mass as these are an ill-defined combination of lean mass, fat mass and height. This potential confusion is highlighted by the contradictory literature on the relationship between obesity and bone mass in childhood, with some studies reporting normal or increased bone mass in obese children (208,309-311), while others conclude that obese children have reduced bone mass relative to their body weight (312,313).

Lean mass

Studies investigating the association between lean mass and bone mass tend to use DXA, as this technique is good at measuring both these constituents of body composition. All observational studies found during review of the literature reported that lean tissue mass is a major predictor of volumetric bone density (204,314-318) in children and adolescents and this has been confirmed by a recent meta-analysis (319). Lean tissue mass is also reported to be positively associated with bone size in childhood (204,320). Proposed mechanisms to explain the association between lean tissue and bone mass in childhood include the action of muscle on bone resulting in loads and strains (315), and genetic factors which explain at least 50% of the covariance between lean mass and bone mass (321).

Fat mass

The literature on the association between fat mass (adipose tissue) and bone mass in children is contradictory. The relationships between fat mass and bone mass is complicated by the fact that children with higher fat mass will also have higher lean mass to carry the extra weight (311). A summary of the literature on the association between fat mass adjusted for lean mass and bone mass in children is shown in Table 13 on page 91. Four studies show a positive association between fat mass and volumetric bone density after adjusting for lean mass (317,318,322,323), and two report no association (311,324). Three studies assess the effects of fat mass on bone size with contradictory results (211,318,324).

Potential explanations for a positive association between fat mass and bone mass include a direct stimulation of bone growth via the mechanical action of increased load because of increased weight (325), by an indirect action on timing of pubertal events as obese children enter puberty earlier than non-obese children (141), artifacts of the systemic errors inherent in DXA methodology that produces a BMC increase when fat adjacent to bone increases (326), or hormonal action of fat (see below).

Several potential mechanisms exist whereby fat mass might exert a negative influence on bone mass in childhood. For example, adipose tissue is known to express aromatase enzymes that convert steroid precursors to oestrogen, which suppress periosteal bone growth (181). Furthermore, increased leptin levels secondary to higher fat mass have been suggested to mediate the negative association between fat mass and periosteal growth observed at non-weight bearing sites (211). A further explanation is that there may be a reciprocal relationship between the development of bone and fat with the nuclear receptor peroxisomal proliferator-activated receptor gamma (PPARγ) being a key transcription factor that may control this (327).

Height

Increasing height during childhood results in increased bone size, i.e. increased length of long bones and increased height of vertebral bodies (328,329). The association between height and volumetric density is less well characterised in the literature because tall children will also tend to have greater lean mass and fat mass than shorter children. No studies were found which specifically investigated the association between height and bone density adjusted for lean and fat mass.

Table 13: Summary of the literature on the association between fat mass adjusted for lean mass and bone mass in childhood

Author, year of publication	Age of participants	No in study	Measure of fat mass used	Measure of volumetric bone density used	Measure of bone size used	Result
Lorentzon et al, 2005, Sweden (211)	18 year old boys	1068	total body adipose tissue by DXA, adjusted for lean mass		cortical CSA and periosteal circumference of radius, and cortical bone size of tibia by pQCT	Negative association between fat mass and cortical CSA and periosteal circumference of radius. Positive association between fat mass and cortical bone size of tibia.
Wang et al, 2005, Finland (322)	10-13 year old girls	258	total body fat mass by DXA adjusted for regional lean mass	size-adjusted BMC of upper and lower limbs by DXA		Stronger positive association between fat mass and estimated volumetric bone density at the lower limb than at the upper limb, but still a positive association at the upper limb. Change in fat mass was positively associated with change in estimated volumetric density at the lower limbs but not the arms.
Pollock et al, 2005, USA (324)	7 year old girls	DK	total body fat mass by DXA, adjusted for lean mass	height-adjusted total body, forearm, femur and L spine BMC by DXA	total body bone area and hip structural measures using DXA	No association between fat mass and estimated volumetric density. Fat mass was positively associated with forearm bone area.
van Langendonck et al, 2004, Belgium (318)	9 year old girls	42	total body fat mass by DXA, adjusted for height	height-adjusted total body, L spine and femoral neck BMD by DXA	bone area of L spine, femoral neck and total body by DXA	Positive association between fat mass and estimated volumetric density at total body only. Positive association between fat mass and bone size at femoral neck and total body but not L spine.
Pietrobelli et al, 2002, Italy (317)	5-17 years	133	total body fat mass by DXA, adjusted for lean mass	lean mass-adjusted total body BMC by DXA		Positive association between fat mass and estimated total body volumetric density
Martinez et al, 2002, Spain (323)	14-17 year old girls	160	total body fat mass by DXA	total body BMC to lean mass ratio by DXA		Positive association between fat mass and estimated volumetric density
Manzoni et al, 1996, Italy (311)	5-18 years	115	total body fat mass by DXA, adjusted for lean mass	weight- and height-adjusted total body BMC by DXA		No association

Abbreviations: BMC bone mineral content; BMD bone mineral density; CSA cross-sectional area; DK don't know; DXA dual energy X-ray absorptiometry; pQCT peripheral quantitative computed tomography; USA United States of America

3.2.3.4. Physical activity

Volumetric density

There is a general consensus in the literature, from both observational studies (135,164,330-338) and interventional studies (339-342) that weight-bearing physical activity during childhood increases volumetric bone density in the bones that are involved in the exercise. There is conflicting evidence on the effects of physical activity on volumetric density of the lumbar spine.

Bone size

The association between physical activity and bone size in childhood is more confusing. Three observational studies (164,331,343) and one interventional study (344) report a positive association between exercise and cortical thickness in childhood, two studies show no effect on cortical thickness (336,341), and one interventional study reported reduced cortical thickness in the intervention group (345). Three observational studies report a positive association between exercise and total body bone area (332,334,337), but one reports no association (268). In conclusion, the literature is contradictory and no firm conclusions can be drawn on the association between physical activity and bone size in childhood.

Interaction between physical activity and puberty

It is not clear whether timing of exercise in relation to puberty has differing effects. One observational study suggests starting regular exercise whilst prepubertal results in greater gains in volumetric density than starting after puberty (330), whereas an RCT reported no effect of the intervention in prepubertal girls, but a good effect in girls in early puberty (346). There is also a suggestion that polymorphisms in the gene that codes for the oestrogen receptor may interact with physical activity in girls (164), such that heterozygotes for a particular polymorphism benefit most from exercise. This evidence comes from one study on 245 10 to 13 year old girls, was not a pre-specified hypothesis, and has not been confirmed in other studies.

3.2.3.5. Drugs and medications

As in adults, a large number of studies have reported an association between childhood oral glucocorticoid use and reduced volumetric bone density (240,347,348). This association appears to be independent of the underlying disease, and the biochemical effects of glucocorticoids on bone are well characterised (348). It is less certain whether inhaled corticosteroids prescribed during childhood for the management of asthma are associated with low bone mass. A large study using the General Practitioner Research Database (GPRD) (349) looked at 97,387 children taking inhaled steroids, and reported no association with fractures after adjustment for asthma severity. Other medications which have been reported to be associated with low bone mass in childhood include warfarin (350) and anti-convulsants (351-353).

3.2.3.6. Summary of potentially modifiable determinants of childhood bone mass

Dietary vitamin D and calcium have a modest positive association with volumetric bone density. Physical activity increases the volumetric density of the bones involved in the exercise. Lean mass is a major predictor of both volumetric bone density and bone size, while the association with fat mass is unclear. Oral corticosteroids reduce volumetric bone density in childhood.

3.2.4. Concomitant diseases that influence bone mass in childhood

The previous sections discussed the non-modifiable, early life and potentially modifiable determinants of bone mass in childhood. This section covers concomitant diseases and is divided into childhood diseases that result in paralysis or inflammation, anorexia nervosa and other diseases

3.2.4.1. Diseases involving paralysis

Many studies report an association between diseases that result in long-term reduced mobility in childhood and low volumetric bone density (353-358). The diseases studied include cerebral palsy, spinal cord injury and other not-clearly specified handicaps. Similar results were seen in a study using QUS (359). One study also reported on reduced bone size in these children compared with normal controls (353). One study looked at biochemical markers of bone turnover and found increased bone resorption but normal levels of bone formation (357). This association between paralysis and reduced bone mass correlates with the severity of the neurological impairment (356), suggesting this is the result of limited mobility. Another potential mechanism for low bone mass in this group of children is the use of anti-convulsant medication to treat associated epilepsy (353,354,356) (see section on medications, page 93).

3.2.4.2. Diseases caused by inflammation

Children with inflammatory conditions such as Juvenile Idiopathic Arthritis (JIA) or Inflammatory Bowel Disease (IBD) have several risk factors that can impair bone health apart from inflammation, such as malnutrition, inactivity, hypogonadism and glucocorticoid use. Inflammation itself is thought to affect bone by production of bone-resorptive inflammatory cytokines (360). Most studies agree (360-364) that children with inflammatory conditions do not have reduced volumetric bone density, but have reduced muscle mass i.e. their bone mass is correct for their lean mass. One study was found that investigated the effects of inflammation on bone size in children and reported reduced cortical thickness in the inflammatory group (362).

3.2.4.3. Anorexia nervosa

Adolescent girls with anorexia have been studied with DXA and bone turnover markers. Results showed consistently lower estimated volumetric bone density (365-367) and lower markers of bone formation (365,366) in girls with anorexia nervosa indicating a low bone turnover state. The pathogenesis of bone deficits in people with anorexia nervosa is likely to be multifactorial and include low body weight, inadequate calcium, vitamin D and protein intake as well as alterations in hormone levels.

3.2.4.4. Other diseases

The literature on the association between other diseases and low childhood bone mass is based on mainly case reports, such as for sickle cell disease (368), and after treatment for acute leukaemia (369). A larger body of literature exists on children with transplants, particularly renal transplants, where the potential risk factors for low bone mass include pre-existing renal osteodystrophy, delayed growth and development, malnutrition, decreased weight-bearing activity, inflammation and immunosuppressive therapy (370). However, the results are conflicting due to lack of adjustment for body size (370-374).

3.2.4.5. Summary of the association between concomitant diseases and bone mass in childhood

Concomitant diseases can have adverse effects on bone quality through a variety of mechanisms including reduced mobility, inflammation, malnutrition, hypogonadism, low body weight and the use of medications such as glucocorticoids or anti-convulsants.

3.3. SUMMARY OF DETERMINANTS OF BONE MASS IN CHILDHOOD

- Bone mass in childhood increases with age, with an acceleration in accumulation between ages 11 and 15 years in girls, and between 13 and 17 years in boys

- Boys have bigger sized bones than girls, and black children have a greater volumetric bone density than white children of similar developmental stages

- Puberty plays a fundamental role in the acquisition of bone mass, acting via multi-hormonal pathways

- Parental size, both at birth and as an adult is associated with higher childhood bone mass

- Maternal diet during pregnancy makes a relatively small contribution towards bone mass in childhood, with calcium intake appearing to be important for volumetric bone density

- Gestational age is positively associated with volumetric bone density of neonates

- Size at birth account for around 70% of the variability in neonatal bone mass

- Dietary vitamin D and calcium during childhood have a modest positive association with volumetric bone density

- Increased physical activity is associated with increased volumetric density of the bones carrying out the exercise.

- Lean mass is a major predictor of both volumetric bone density and bone size, while the association with fat mass is unclear

- Concomitant diseases have adverse effects on bone quality through a variety of mechanisms

CHAPTER 4: INJURY AND FRACTURE

The previous two chapters have described the structure, growth and properties of bone and reviewed the literature on the determinants of bone mass in children. This chapter focuses on injury and fracture and is divided into four sections. The first section gives an overview of childhood injury epidemiology, the second defines fractures, the third section discusses the identification of fractures, and the fourth focuses on the use of trauma level in childhood fracture epidemiology.

4.1. CHILDHOOD INJURY EPIDEMIOLOGY

4.1.1. 'Injury' versus 'Accident'

The word injury is derived from the Latin '*iniūria*' meaning a wrong or injustice. Today it can be defined as an act that injures someone or something, or as any physical damage to the body caused by violence or accident (375). Injury also has a legal definition: for example in the US it is a violation of the rights of another party for which legal redress is available. The word accident on the other hand, is defined as anything that happens by chance without an apparent cause, or a mishap, especially one causing injury or death (375). Injuries are commonly referred to as 'accidents', but they are not random, unpredictable events (376), and have many predictable factors. Therefore I will use 'injury' in preference to 'accident' in this thesis.

The role of injury epidemiology is to classify and describe injuries, to develop and use injury surveillance systems, to identify risk factors for injuries, and to develop and test injury prevention strategies (377). Traditionally, childhood injury epidemiology is divided according to the circumstances of the injury into submersion injuries (drowning), fires and burns, poisoning/swallowing, falls, road traffic injuries and violence. In terms of fractures, these are likely to occur as a result of falls, road traffic injuries and violence, and so I will focus on these and call them traumatic injuries.

To define exactly what constitutes an injury is complex, as injuries may be defined simultaneously by the causative event and by the resulting pathology (378). For example bruising can occur without any trauma in people with bleeding disorders and in this situation is not an injury. Sometimes the same causative event may cause an injury or a disease. For example, brief exposure to toxic gas may cause an injury to the airways, whereas chronic exposure to low concentrations of the same gas may result in a chronic response that is called disease (9). However, when focusing on traumatic injuries that may result in fractures, the Haddon matrix (described below) provides a useful strategy for definition.

4.1.2. The Haddon matrix

A more detailed way of dividing up the risk factors for injury epidemiology and providing a framework for the definition of traumatic injury was devised by William Haddon in 1968 (379) which he called the 'phase-factor' matrix. The matrix identifies three time periods: pre-event, event and post-event. The matrix then describes the host, the agent/vehicle, the physical environment and the social environment (community norms, policies and rules) in each of these time periods.

Factors contributing to the probability that an injury-producing event will occur in the first place, feature in the *pre-event* stage. Factors that make the outcome of a fracture more likely given that an injury-producing event has occurred, will feature in the *event* stage. Factors affecting the impact of the fracture on the individual's health, psychological balance or social interactions will feature in the *post-event* stage. An example of this matrix applied to a fractured femur sustained by being struck by a car, is given in Table 14, page 99.

4.1.3. Descriptive epidemiology of childhood traumatic injury

Injuries are a leading cause of death and disability among children in the United Kingdom, costing an estimated £200 million annually in direct costs to the NHS (380).

In 2002 the rate of death per 100 000 children aged 0 to 14 years due to injury or poisoning was 4 for males and 3 for females (381). However, deaths are just the tip of the iceberg; nonfatal injuries to those under the age of 20 lead to approximately 10 million A&E visits and more than 10 million primary care office visits in the US each year (14). Injuries to children have tolls in terms of deaths, hospitalisations, A&E visits, and also have an economic impact: injuries are the leading cause of medical spending for children aged 5-14 years of age (14).

Table 14: Example of a Haddon matrix for the risk-factors and outcome of a fractured femur in a 7 year old child, occuring as a result of being struck by a car.

	Human Factor (Host)	Agent/vehicle	Physical environment	Social environment
Pre-event (factors contributing to the probability that an injury-producing event will occur in the first place)	•Age •Lack of experience crossing the road	•Speed of car	•Busy road •No dedicated crossing area •No street lights	•Road safety laws •Speed cameras •Speed bumps
Event (factors that make the outcome of a fracture more likely given that an injury-producing event has occurred)	•Bone density / fragility •Padding e.g. obesity, clothing	•Presence of 'bull-bars'	•Road surface	•Banning certain kinds of car bumpers and 'bull-bars' •Banning off-road vehicles in towns
Post-event (factors affecting the impact of the fracture on the individual's health, psychological balance or social interactions)	•Type of fracture e.g. open or closed •Age •Physical condition		•Quick response by ambulance •Dedicated paediatric A&E or trauma departments •Experienced paediatric orthopaedic surgeons	•Political support for dedicated paediatric trauma centres •Funding for more front-line ambulances

Abbreviations: A&E Accident and Emergency department

Death rates in children due to traumatic injury or poisoning are declining. A study of Scottish children (382) showed the death rate from injury or poisoning in children aged 0-14 years fell from 13.2 per 100 000 during 1981 to 1993, to 7.4 per 100 000 during 1993 to 1995. However, when traumatic injuries were looked at in isolation, the proportion of deaths decreased only slightly, from 14% of all deaths in children in 1981 to 1983 to 12% in 1993 to 1995.

In terms of age, there is a bimodal distribution in injury death rates for children and teenagers. Young infants are at higher risk of inflicted trauma due to their small size and inability to protect themselves. Whereas risks for teenagers are increased as a result of increased exposure to hazards such as road travel, and an increase in risk taking behaviour such as drinking alcohol (14). In injury epidemiology 'hazard' means the 'inherent capability of an agent or a situation to have an adverse effect, or a factor or exposure that may adversely affect health' (377). It is similar to the concept of a 'risk factor' that can be defined as 'an aspect of personal behaviour or life-style, an environmental exposure, or an inborn or inherited characteristic that, on the basis of epidemiologic evidence is known to be associated with health-related condition(s)' (383).

Many analyses and research studies consistently show that male gender is a risk for all types of injury (7,14,384-389). Rates of fatal and non-fatal traumatic injury are also higher in children of a lower social position (382).

4.1.4. Epidemiology of childhood injury due to falls

The types of injuries that are more likely to result in fractures are traumatic injuries: falls, road traffic injuries and violence. This section reviews the literature on childhood injury due to falls. Road traffic injuries are covered on page 102, and childhood injuries due to violence on page 103.

Most of the literature on falls in childhood comes from the US. In New York City, falls are the third leading cause of traumatic death in children under 13 years (385), but childhood mortality from falls is still an uncommon event. However, morbidity from falls is large. Falls are the most frequent cause of injury bringing children to the A&E department (386,387). A summary of the hazards for fracture after a fall in childhood is shown in Table 15, page 101.

4.1.4.1. Pre-event hazards for falls in childhood

The pre-event hazards are those factors that make a fall in childhood more likely. These include younger age (388), male gender (384), lower socio-economic class (390) and living in urban areas (386). Falls are most likely to occur between noon and evening (391). Recent work has suggested that African-American children in the US are over twice as likely to be killed by an injury than white children (389). This study did not explore whether this was explained by socio-economic status.

Table 15: Haddon matrix summarising the risk-factors for childhood fractures as a result of a fall

	Human Factor (Host)	Agent/vehicle	Physical environment	Social environment
Pre-event (factors contributing to the probability that an injury-producing fall will occur in the first place)	•Younger age •Male gender		•Living in an urban area •Time of day	•Lower socio-economic background
Event (factors that make the outcome of a fracture more likely given that an injury-producing event has occurred)	•Bone density / fragility •Padding e.g. obesity, clothing	•Height of fall, particularly if >3 meters	•Landing surface, especially concrete	
Post-event (factors affecting the impact of the fracture)	•Type of fracture e.g. open or closed •Age •Physical condition		•Quick response by ambulance •Dedicated paediatric trauma departments •Experienced paediatric orthopaedic surgeons	•Political support for dedicated paediatric trauma centres •Funding for more front-line ambulances

4.1.4.2. Event hazards for falls in childhood

These hazards are those that make it more likely that an injury will occur as a result of the fall. Stairs, steps, beds, tables and chairs are the most common structure that children fall from (392). However, in terms of injury the *height of the fall* is important. Falls of more than three meters are classed as high-energy trauma and are likely to cause serious injury or death. Falls from half to three meters are classed as medium-energy trauma and may cause serious injury. Falls from standing height or less are

classed as low-energy trauma, and are unlikely to cause serious injury (7). See page 117 for a further discussion of trauma level. In terms of the *landing surface,* Cummins and Potter suggest that the contact surface is more important than the height of the fall, with concrete being particularly unforgiving (393).

4.1.4.3. Post-event hazards for falls in childhood

These will be the same as for all types of injury in childhood. The 'human factors' will include the ability of the child injured to recover and this will depend partly on any pre-existing illnesses or health conditions. Proximity to medical care will also be important in recovery from injury. In some countries, notable the US, having or not having Health Insurance may also affect recovery (394).

4.1.5. Epidemiology of childhood injury due to road traffic injuries

No other injury event takes the lives of more children than motor vehicle crashes (381). Injuries to children occur as a pedestrian, as a bicycle user or as a passenger in a car. Among children aged 5 to 9 in the US, pedestrian injuries are the most common cause of motor vehicle fatalities (378). In bicycle injuries that result in childhood death, around 89% involve a crash with a car (14). Bicycle injuries in children are more likely to be due to a mistake made by the child themselves, whereas in adult bicycle injuries, the mistake is usually due to the car driver (395).

4.1.5.1. Pre-event hazards for road traffic injuries in childhood

The pre-event hazards are those factors that make a motor vehicle crash more likely. In a demographic study of childhood pedestrian injuries in Memphis, USA (396), the injured child was most often male and had an average age of 7.3 years. The child was usually struck while crossing the road between intersections, most commonly between the hours of 2 to 7 pm. In this same study, the areas with highest child pedestrian injuries had twice the percentage of non-white population, lower household incomes, more children living in female-headed households, more families living below the poverty level, and greater household crowding (396).

4.1.5.2. Event hazards for road traffic injuries in childhood

These hazards are those that make it more likely that an injury will occur as a passenger, as a result of the motor vehicle crash. The major hazards for childhood death in the result of a crash is the failure to use a safety restraint device. However, the type of device needs to be appropriate for the age and size of the child (397). Teenagers, the group most at risk of death from a motor vehicle crash, are least likely to wear a seatbelt (14). For children riding bicycles who are involved in a road traffic crash, head injury is the greatest risk. A Cochrane Review concluded that the use of bicycle helmets reduce bicycle-related head and facial injuries for bicyclists of all ages involved in all types of crashes, including those involving motor vehicles (398). No literature was found investigating the association between car design features such as air-bags, bull-bars or crumple zones and injuries in children.

4.1.5.3. Post-event hazards for road traffic injuries in childhood.

These will be the same as for all types of injury in childhood - see paragraph 4.1.4.3, page 102.

4.1.6. Epidemiology of childhood injury due to violence

Violence resulting in injury or death to children includes physical abuse and suicide.

4.1.6.1. Physical abuse as a cause of injury in children

Physical abuse is a leading cause of infant death (9.1 per 100 000 births in the US) and serious head injury in young children (399). Pre-event hazards for abuse are reported to be low socio-economic status, children in families with unstable social situations, children born prematurely, or children with disabilities (400). Event and post-event hazards, which affect the recognition and therefore the outcome of abuse, include experience and recognition of abuse by health personnel. Missed diagnoses are most likely in younger white children, and those from two-parent households (401).

4.1.6.2. Suicide or attempted suicide as a cause of injury and death in children.

Suicide is often a neglected childhood and teenage problem. In the US, suicide mortality rates rise during the preteen years, and then rise again sharply in the 15 to 24 year old age range (14). Suicide is the third leading cause of death for the 10 to 24 year old age

group in most of the US (402). It is estimated that for every completed suicide, 100 to 200 attempts are made (14). Pre-event hazards for suicide in children include being male, having been arrested in the past, and drug and alcohol abuse (403). Event and post event hazards which increase the risk of suicide resulting in death include gun ownership in the family home (403,404).

4.1.7. Summary

Traumatic injuries in childhood such as falls, road traffic injuries, violence, physical abuse or attempted suicide may result in bone fractures. Factors contributing to the probability that an injury-producing event will occur in the first place (pre-event hazards), factors making a fracture more likely given that an injury has occurred (event hazards) and factors affecting the impact of the fracture on the child's health (post-event hazards) can all be described in a Haddon matrix.

Pre-event factors that are common to all types of traumatic injury that may result in fractures, are being male and having a lower socio-economic background. Event factors that make it more likely for a fracture to occur vary depending on the type of traumatic injury. Height and landing surfaces are important in falls, whereas the use of safety devices such as seat-belts or bicycle helmets can affect the likelihood of fracture in road traffic injuries. Post-event factors are common to all and include any pre-existing illnesses or health conditions.

4.2. DEFINITION OF FRACTURES

Appley's System of Orthopaedics and Fractures (405) defines fractures as 'a break in the structural continuity of bone. It may be no more than a crack, a crumpling or a splintering of the cortex [but] more often the break is complete and the bone fragments are displaced'.

Fractures of bone vary in appearance, but in general may be divided into a few well-defined groups. Incomplete fractures occur most often in children. Here, the periosteum remains in continuity, and the bone is simply buckled or bent (see Figure 13). In complete fractures, the bone is broken into two or more fragments (see Figure 14). These fragments of bone may remain roughly in place, be displaced, or be impacted i.e. pushed tightly together.

Figure 13: X-ray of the forearm of a child showing an incomplete (greenstick) fracture of the radius and ulna where the bone is simply bent.

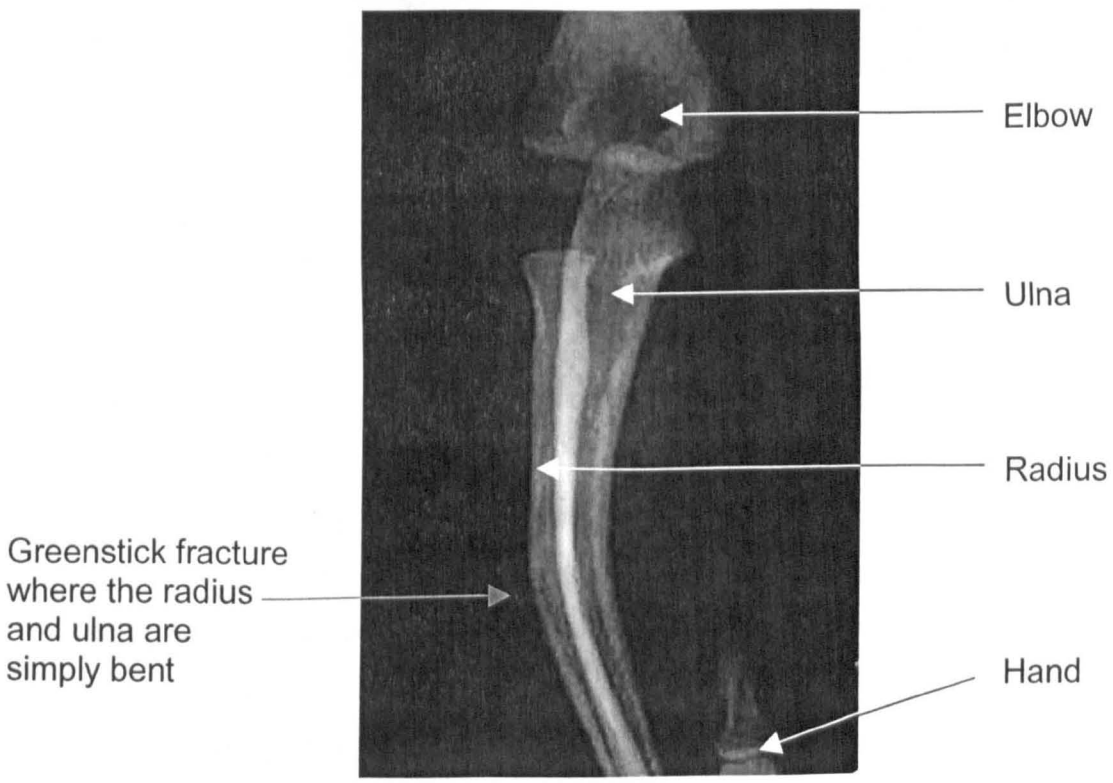
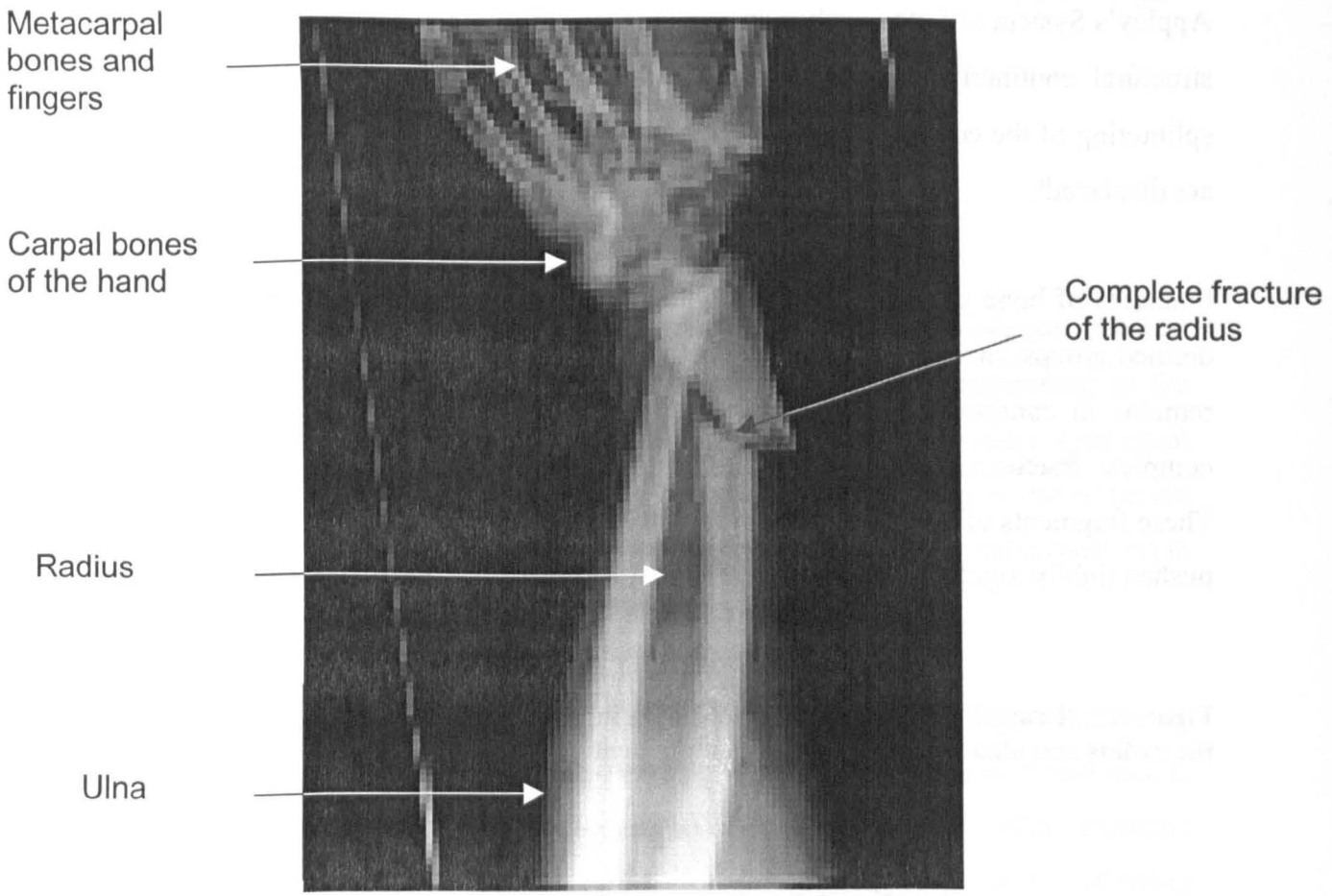


Figure 14: X-ray of the forearm of an adult showing a complete fracture of the radius. Reprinted with kind permission from Dr Timothy J Biega - image available from MedPix.



4.3. IDENTIFICATION OF FRACTURES

This section looks at how individuals who sustain fractures are identified. Identification of fractures in individuals is important in the acute situation to ensure the appropriate management is undertaken, to minimise morbidity and long-term dysfunction (406). However, for epidemiological research it is also important to be able to identify individuals within populations who have had a fracture in the past.

4.3.1. Diagnosis of fractures in individuals in the acute situation

In most acute situations the account of the injury (history of the injury) will make the diagnosis of a fracture likely e.g. 'I heard something snap when I landed on my outstretched arm, and now my arm looks bent' (405). This can then be confirmed by taking an X-ray of the affected area. While the most frequently used form of medical imaging for suspected fractures is the plain radiograph or X-ray, there can be diagnostic difficulties with fractures in particular parts of the body, where plain X-rays can be difficult to interpret. In these situations other imaging techniques such as Magnetic Resonance Imaging (MRI) may be used.

4.3.1.1. History

As discussed above, often the history given will be clear enough to suspect a fracture. However, this is not always the case. For example, the description of an injury causing an avulsion fracture of the ischial tuberosity in adolescents (usually whilst doing the splits) can closely mimic that of a hamstring injury, such that X-rays are not performed and the fracture is missed (407). Avulsion fractures of the ischial tuberosity are described as 'rare' (408), although no estimates of prevalence are available. Sometimes an individual will be unable to provide the history themselves, for example a young child. In this case, unless the injury was witnessed, the history will be unreliable (405).

4.3.1.2. Clinical examination

Clinical examination to diagnose fracture can be useful when the history of injury is uncertain, but imaging techniques should also be performed to see if surgical

intervention is required. A meta-analysis of 12 studies (409) investigating the sensitivity and specificity of the clinical examination for detecting pelvic fractures in adults found 49 missed pelvic fractures out of a total of 441, indicating an 11% false negative rate. The relevance of this study to more common fractures such as fractured distal forearm is not clear, but it seems likely that limb fractures are easier to diagnose on examination.

4.3.1.3. Plain radiographs or X-rays

An X-ray is the most common and usual investigation when a fracture is suspected (410). Correct interpretation of the X-ray is vital: one study of junior doctors in A&E showed that only 82% of fractures were correctly identified on the day of attendance (411). Another study looking at acute cervical spine injuries in infants and children also found that only 81% of fractures were correctly identified by doctors during the initial emergency room evaluation (412). In this later study there was a 5% false negative rate and a 14% false positive rate. The most common factors associated with a misdiagnosis of fracture were unfamiliarity with paediatric cervical spine anatomy, failure to recognise normal variants seen during growth and development, and sub-optimal X-ray film techniques.

Some fractures can be difficult to identify with plain X-rays. One of the most common is a fracture of the scaphoid. The scaphoid bone is one of the carpal bones of the hand, and up to 25% of these fractures are initially not visible on plain X-rays (413). For suspected scaphoid fractures where the X-ray appears normal, the usual management in the United Kingdom is to put the forearm in plaster and repeat the X-ray in 2 weeks. Alternative management can include other imaging techniques such as ultrasound or MRI. Other fractures that can be difficult to detect on plain X-rays include fractures of the nose (414), the temporal skull bone (415), the ischial tuberosity (407), the hook of the hamate (another carpal bone) (416) and fractures that run across the growth plates of long bones (417). However, the most common fractures in children (distal forearm) are easily diagnosed with a plain X-ray.

4.3.1.4. Magnetic Resonance Imaging (MRI) and other techniques

MRI scans are the investigation of choice in an individual with a suspected scaphoid fracture when the plain X-ray appears normal (418). These scans produce images by using a strong magnetic field to line up the abundant hydrogen nucleus's found in fat

and water (419). See page 47 for a further discussion of MRI. There are no known biological hazards of MRI because it does not use ionising radiation. Ultrasound for diagnosis of paediatric fractures is currently a research tool (420), but may be used more in the future because it does not use ionising radiation to produce the image.

4.3.2. Identification of people within populations who have had a fracture

The previous section described the diagnosis of fractures in the acute situation. For epidemiological research it is also important to be able to identify individuals within populations who have had a fracture in the past. This section describes the literature on the identification of individuals with a history of fractures by looking at previously collected data or by new data collection. Very few studies were found that looked specifically on the identification of children with a history of fractures.

4.3.2.1. By use of previously collected data

Sources of routine data are used to produce descriptive statistics and surveillance of a health outcome, and can also be used as a resource for analytical epidemiological studies. Their usefulness depends on their completeness, accuracy and consistency.

As this thesis is based on a study carried out in the UK I have focussed on routine data available on fractures occurring in the UK. In the UK, routine sources of data on fractures in children can be found as part of the Home and Leisure Accident Surveillance Systems (HASS and LASS), National Study of Morbidity Statistics from General Practice and the General Practice Research Database (GPRD). All of these databases use samples of the UK population to collect data on fractures in children. National estimates or prevalence rates are then produced by using data from UK-wide censuses or population registers for the denominator. This has limitations, as census data tends to be incomplete for population subgroups such as the homeless or very young children, and can rapidly become out of date (421). However, use of census data for the denominator is the best method available.

Home and Leisure Accident Surveillance Systems(422)

Data from the Home Accident Surveillance System (HASS) and the Leisure Accident Surveillance System (LASS) are available for 1978 to 2002. They were set up to gain an

in-depth understanding of how and why home and leisure injuries occur, to enable the Department of Trade and Injury (DTI) to take steps to prevent them from happening in the future. HASS and LASS contain records of non-fatal injuries occurring in the home or at leisure, which resulted in an injury serious enough to warrant a visit to hospital. The data is based on attendance records of a sample of 13 to 18 UK hospitals with a 24 hour Accident and Emergency department recording at least 10 000 cases per year. The injuries were collected from attendance records, and further details were obtained by interviewing the patient or person accompanying them.

HASS and LASS provide data on children aged 0-4 and 5-14 years who had a 'bone injury' during a specific year between 1978 and 2002 and who presented to one of the 18 hospitals included. Bone injury is defined as either a fracture (suspected, open, closed or unspecified) or a crush injury. National estimates have been produced for all injuries in a certain age group, or for bone injuries in the whole population. National estimates have not been produced for bone injuries in sub-populations such as children.

Morbidity Statistics from General Practice (9)

It was recognised in the 1950s that the patterns of GP consultations could provide useful indicators of the general health of the nation. Prevalence trends in sickness, including the increasing or decreasing incidence of particular conditions, could help to inform health service practitioners and planners. The first study (National Morbidity Statistics 1: NMS1) took place in 1955-6, and further studies took place in 1970-6 (NMS2) and 1981-2 (NMS3).

The fourth national study of morbidity statistics from General Practice was commissioned by the Department of Health and carried out between September 1991 and August 1992. Its objectives were to examine the pattern of disease seen by GPs by the age, sex and socio-economic status of the patient, and to provide information to those planning health care resources.

This study covered a one percent sample of the population of England and Wales (502,493 people registered with GPs). These people were selected from the NHS lists of 60 GP practices which volunteered to take part. Data were collected using specially designed software on computers in the GP practices and transferred to the Office of

Population Censuses and Surveys (OPCS) on discs. The fourth National Study of Morbidity Statistics from General Practice represents the best data currently available on morbidity seen in general practice.

In the section on injuries data are available on the incidence rates per 10 000 person years at risk for fractures of the femur, humerus, radius and ulna, and rib for boys and girls aged 0-4 and 5-15 (see Table 16, below). Data area also available on the number of new and first ever episodes (see Table 17, below), consultations with doctors or consultations with nurses for all types of fracture for children.

Table 16: Incidence rates per 10 000 person years at risk, by sex, for fractures of the femur, humerus, radius/ulna and rib for children. From the 4th National Study of Morbidity Statistics from General Practice (9)

	Aged 0-4 years		Age 5-15 years	
	Males	**Females**	**Males**	**Females**
Femur	0	0	0	0
Humerus	2	4	6	6
Radius and ulna	12	14	35	26
Rib	1	1	2	1

Table 17: Doctor consultation rates per 10 000 person years at risk for children with fractures of the skull, spine/trunk, upper limb or lower limb. From the 4th National Study of Morbidity Statistics from General Practice (9)

	Aged 0-15 years
Skull	7
Spine and trunk	2
Upper limb	62
Lower limb	23

General Practice Research Database (GPRD)(423)

The GPRD is owned by the Department of Health and managed by the Medicines Control Agency (which is now part of the Medicines and Healthcare Products Regulatory Agency: MHRA). It contains the anonymised longitudinal computerised medical records of almost 400 general practices in the UK. The data collected includes

demographic information about the people registered with the practice, prescription details, clinical events, preventative care provided, referrals to specialist care, hospital admissions and their major outcomes. The diseases or causes of morbidity and mortality are cross-referenced to the International Classification of Diseases, ninth edition (ICD-9). Several independent validation studies have shown that the database has a high level of completeness and validity (424,425). Access to the database has financial implications, depending on the amount of data required e.g. £300 000 per annum for full access. The data from the GPRD is different to the data available from the General Practice Morbidity Statistics, as it doesn't just provide data on consultations, but can be used to derive incidence rates.

A study by Cooper et al using this database (2) found a total of 52 624 boys and 31 505 girls aged <18 years fractured a bone over an 11-year follow-up period. This gives a rate of 133.1/10 000 person years. Table 18 below is adapted from this study and shows the distribution of fractures and incidence rates for childhood fractures collected using the GPRD.

Table 18: Distribution of fractures and incidence rates standardised to the UK population per 10 000 person years for boys and girls < 18 years. Data from the GPRD, adapted from the study by Cooper et al (2).

	Boys		Girls	
	Number	Rate per 10 000 py	Number	Rate per 10 000 py
All Fractures	52 624	161.6	31 505	102.9
Upper limb	39 502	121.3	23 793	77.7
Clavicle	4 672	14.5	2 287	7.6
Lower limb	10 749	33.0	6 189	20.2
Skull	3 393	11.3	1 404	5.3
Ribs	233	0.8	106	0.4
Vertebral	159	0.5	134	0.4
Pelvis	125	0.4	89	0.3

Abbreviations: GPRD General Practice research database; py per year

4.3.2.2. By new data collection

The previous section detailed how routine data can be used to identify children with a history of fractures from populations. This section focuses on identification by new data collection.

Cohort studies collecting fractures from A&E records

Two cohort studies have been found that collected data on incidence rates of fractures in childhood: one from South Wales (3) and one from Norway (1). These two cohorts use the same study design and were carried out by the same author (R Lyons). The study design was to enrol all hospitals within a defined catchment area and obtain weekly data on all fractures occurring in children aged 0-14 years. Mapping of postcodes was used to determine residency within the geographical area. Details were collected on where the injury occurred, what the person was doing at the time of the injury, what went wrong, and on what did the person injure themselves. Where data were missing, parents and children were followed up via telephone calls. Data were amalgamated with census data for the catchment area. This study design has also been implemented in Finland, Sweden, Latvia and Croatia but data from these last four countries are not yet available.

One of the main aims of these cohort studies was to produce incident rates for various types of childhood fractures. The results show that while South Wales appears to have many more childhood fractures than Norway, fractures of the upper limb are most common in both South Wales and Norway. However, while fractures of the radius and ulna are more common in Wales, fractures of the scaphoid are the most common fracture type in Norway. A summary of the results of the two studies is shown in table 19 on page 114. Possible reasons for the higher incidence of fractures in South Wales is discussed further in the next chapter on the determinants of fracture risk in children, page 122.

Table 19: Incidence rates of fracture per 1000 children collected from 2 contemporary cohorts

	Incidence rate of fracture per 1000 children per year	
	S Wales: aged 0-14 in 1996 [3]	Norway: aged 0-14 in 1996 [1]
Radius/ulna	13.0	2.1
Fingers	5.1	1.7
Humerus	2.6	0.9
Clavicle	2.2	1.6
Carpal bone including scaphoid	1.9	4.3
Tarsus/metatarsus	1.7	1.4
Toes	1.5	0.9
Ankle	1.3	-
Face/nose	1.1	0.6
Tibia/fibular	1.0	1.0
Skull	0.7	0.2
Femur	0.3	0.3

Questionnaires

No studies were found that looked specifically at the accuracy or precision of questionnaires to identify individuals within populations as having had a previous fracture. No studies were found that looked at parents answering questionnaires about fractures in their children.

One study (426), as part of the European Prospective Osteoporosis Study, looked at the validity of a postal questionnaire concerning fractures in the elderly, by sending it to 144 men and women aged 50 to 84 years who had been hospitalised within the last 12 months because of a fracture. Of these, 8% of respondents denied any recent fracture. Another study (427) assessed the agreement between self-reporting of a range of diseases during face-to-face interview with community-dwelling disabled women aged 65 and over, and found a Kappa statistic for hip fracture of 0.96. Another study investigated the accuracy of self-administered questionnaires in people attending an orthopaedic outpatient clinic, compared to the medical records, for collecting details on past medical history (428). The mean percentage agreement was 96% (range 57-100%) across all questionnaire items.

Direct interview and self-report

One recent study based on the AGES (Age, Gene/Environment Susceptibility) Reykjavik Study asked 2286 elderly people (aged 66 to 95 years), all who had had a previous fracture, whether they had had a fracture (429). Men were consistently less reliable in correctly answering the question than women, with a Kappa statistic for wrist fractures in men of 0.51 compared with 0.78 for women. Those who incorrectly reported no previous fracture were more likely to be cognitively impaired. The study concluded that self-report by adults was relatively accurate when ruling out fractures. However, the authors concluded self-report was less useful as a positive predictor for previous fracture, and suggested the need for ascertainment of fractures based on radiographs and medical records in epidemiological research.

X-ray reports

One interesting study looked at the probability of fracture among American children aged 6-16 years referred for an X-ray after an injury (430). They found that boys were more likely than girls to have a fracture identified in the radiology report (64.7% versus 52.0%, $P<0.001$), and whites and other races were more likely than blacks to have a fracture identified (62.2% and 61.4% versus 49.5%, $P<0.001$).

4.3.3. Summary

In the acute situation, plain X-rays will give the correct diagnosis of fracture in the majority of situations, particularly if they are reported by a specialist. In rare occasions other imaging modalities such as MRI may be necessary.

For epidemiological purposes, people within populations who have had a previous fracture can be identified through routine data collection, or by collection of new data. Routine data collection in the UK that allows identification of people with fractures includes HASS, LASS and the GPRD. Little information is available on new data collection specifically with the aim of identifying children with fractures, but data from A&E has been used. In adults, postal questionnaires can correctly identify approximately 92 to 96% of people with previous fractures.

There is limited literature on identification of individuals who sustain fractures. Despite this, the evidence suggests that most fractures present in a straightforward manner, and physical examination combined with plain X-ray usually confirm the diagnosis and guide treatment.

4.4. USE OF TRAUMA LEVEL IN CHILDHOOD FRACTURE EPIDEMIOLOGY

In the childhood fracture epidemiology literature only one method is used to describe the injury that resulted in a fracture in terms of the level of trauma experienced. This was first used by Landin (7) in 1983 and is a classification method based on descriptions of the events surrounding the injury including height of falls, type of activity engaged in and the use of any equipment. Injuries are categorised into slight, moderate or severe trauma.

4.4.1. Categorisation of injuries

4.4.1.1. Slight trauma

Low trauma injuries are described as those caused by forces exerted by the injured individual. Typical examples of slight trauma injuries are shown in Table 20, below.

Table 20: Examples of Landin's description of slight trauma injuries

• Falling to the ground from standing on the same level
• Falling from less than 0.5 metres e.g. falling from stools, chairs or beds
• Playing injuries including playground scuffles
• Low energy sporting injuries such as ball sports, skating, wrestling, judo, karate and gymnastics
• Skiing, skateboard and roller skating injuries

4.4.1.2. Moderate trauma

Typical examples of moderate trauma injuries according to Landin's description are shown in Table 21, below.

Table 21: Examples of Landin's description of moderate trauma injuries

• Falling from between 0.5 and 3 metres
• Falling from a bunk bed
• Baby being dropped to the floor by an adult
• Falling downstairs
• Falling from a bicycle
• Falling from horse-back
• Falling from swings or slides or similar playing equipment
• Child being hit by a bicycle

4.4.1.3. Severe trauma

Typical examples of severe trauma injuries according to Landin's description are shown in Table 22, below.

Table 22: Examples of Landin's description of severe trauma injuries

• Falling from a height exceeding 3 metres • Most falls from windows or roofs • All traffic injuries excluding being hit by a bicycle • Being hit by a moving heavy object

4.4.2. Association between injury trauma levels and fractures in children.

In Landin's paper (7) based on an analysis of 8,682 fractures in Swedish children between 1950 and 1979, data were only available on injuries that resulted in fractures. No data is available on injuries that did not result in fractures for comparison. However, in this descriptive paper, fractures were caused by trauma classified as slight in 66%, moderate in 19%, severe in 7% and not classified or unknown in 8%

4.4.3. Problems with Landin's trauma levels

Landin classified each injury according to information obtained retrospectively and the degree of trauma was difficult to evaluate in many instances and impossible in others e.g. birthing injuries, being caught in doors or involvement with various types of household equipment. This means some injuries cannot be classified according to the above criteria.

Some of the examples in the slight trauma category, particularly skiing, skateboard and roller skating injuries, can be associated with significant degrees of speed that may result in high forces involved in any subsequent fall. These may fit better in the moderate trauma category. No description of landing surfaces are included in the categorisation, and landing surfaces are important factors that make the outcome of a fracture more likely given that a fall has occurred (see page 101).

4.5. SUMMARY OF INJURY AND FRACTURE

- Traumatic injuries in childhood that result in fractures include falls, road traffic injuries and violence

- Risk factors for traumatic injuries can be divided into those contributing to the probability that an injury-producing event will occur in the first place (pre-event hazards); those that make the outcome of a fracture more likely given that an injury-producing event has occurred (event hazards); and those affecting the impact of the fracture on the individual's health, psychological balance or social interactions (post-event hazards)

- The main hazards for injury in childhood are being male and being from a lower socio-economic background

- A fracture is a break in the structural continuity of bone, and may be incomplete (most common in children) or complete

- In the acute situation, plain X-rays will give the correct diagnosis of fracture in the majority of situations

- For epidemiological purposes, people within populations who have had a previous fracture can be identified through routine data collection, or by collection of new data

- Most childhood fractures occur as a result of injuries that involve slight degrees of trauma

CHAPTER 5: DETERMINANTS OF FRACTURES IN CHILDHOOD

The previous chapters have discussed the structure and properties of bone, the determinants of bone mass in childhood and childhood injury epidemiology. This chapter reviews the literature on the determinants of fractures in children, except for bone mass, which is described in detail in the next chapter. This chapter is divided into two sections. The first details the descriptive epidemiology of fractures in childhood, and the second section describes the analytical epidemiology of the determinants of fractures in childhood.

5.1. DESCRIPTIVE EPIDEMIOLOGY OF FRACTURES IN CHILDREN

Fractures in children are an important but neglected public health issue. One (4) to 3.5% (3) of children fracture a bone each year, and the lifetime risk of sustaining a fracture in childhood for boys is 42-64% and for girls is 27-40% (4,7). Fractures in childhood are not a benign condition as they impact on daily activities. For example, the mean number of activity restricted days for leg fractures in children aged 0-12 years in Seattle, USA is 26 days (95%CI 7 to 45), and for arm fractures is 14 days (95%CI 8 to 20) (12). Other adverse outcomes of fractures in childhood include mal-alignment of the fractured bone, limb overgrowth (particularly in femoral shaft fractures) (10) and acute compartment syndrome (11). Even 'simple' fractures result in time off school whilst attending Accident and Emergency departments and follow-up appointments in Fracture Clinic. There is also increasing concern (34,91) that children who fracture bones may become adults who fracture bones. As detailed on page 41 postmenopausal hip fractures currently cost the NHS approximately £1.5 billion pounds per year (80). Despite these important consequences of childhood fractures, few studies have investigated the incidence or determinants of fractures in childhood. Childhood fractures need to be

recognised as a public health issue, and not seen simply as accidents determined by fate and consequently not preventable, but as problems to be solved.

Below I present a literature review of the epidemiology of fractures in children focusing on incidence rates, site of fracture in the skeleton, repeat fractures, age, gender, ethnicity and secular trends. I carried out the review using similar methods to those described on page 53. Although I did not use the methods of a rigorous systematic review, I have been reviewing the literature for the past few years, and I believe the information presented below adequately describes the current state of knowledge.

5.1.1. Incidence rates

Most of the studies on fracture epidemiology in children give age-standardized incidence rates. Two large population-based studies on the epidemiology of childhood fractures in Great Britain have been published (2,3). The study by Cooper *et al* published in 2004, looked at age- and sex-specific incidence rates for fractures at various skeletal sites in children aged less than 18 years living in the UK from 1988 to 1998. This study used the General Practice Research Database (detailed on page 111), which has a national coverage of about 6%. The study by Lyons *et al* published in 1999, looked at fracture incidence in children aged 0 to 14 years living in the Swansea and Neath Port Talbot areas of South Wales in 1996.

These two studies reported markedly different rates. In South Wales the fracture rate was 36.1/1000 children (3), whereas using the General Practice Research Database the overall rate for the UK (England, Scotland, Wales and Northern Ireland combined) was 13.3/1000 children-years (2). The geographical breakdown of fracture incidence in the study by Cooper *et al* showed Wales had one of the highest fracture rates (16.3/1000) in children compared to southeast England, but this is almost half the rate reported in the study by Lyons *et al.* These differences are unlikely to be explained by differences in study design alone as both used well-defined population-based datasets, so the underlying reason for the higher rate in South Wales is unclear.

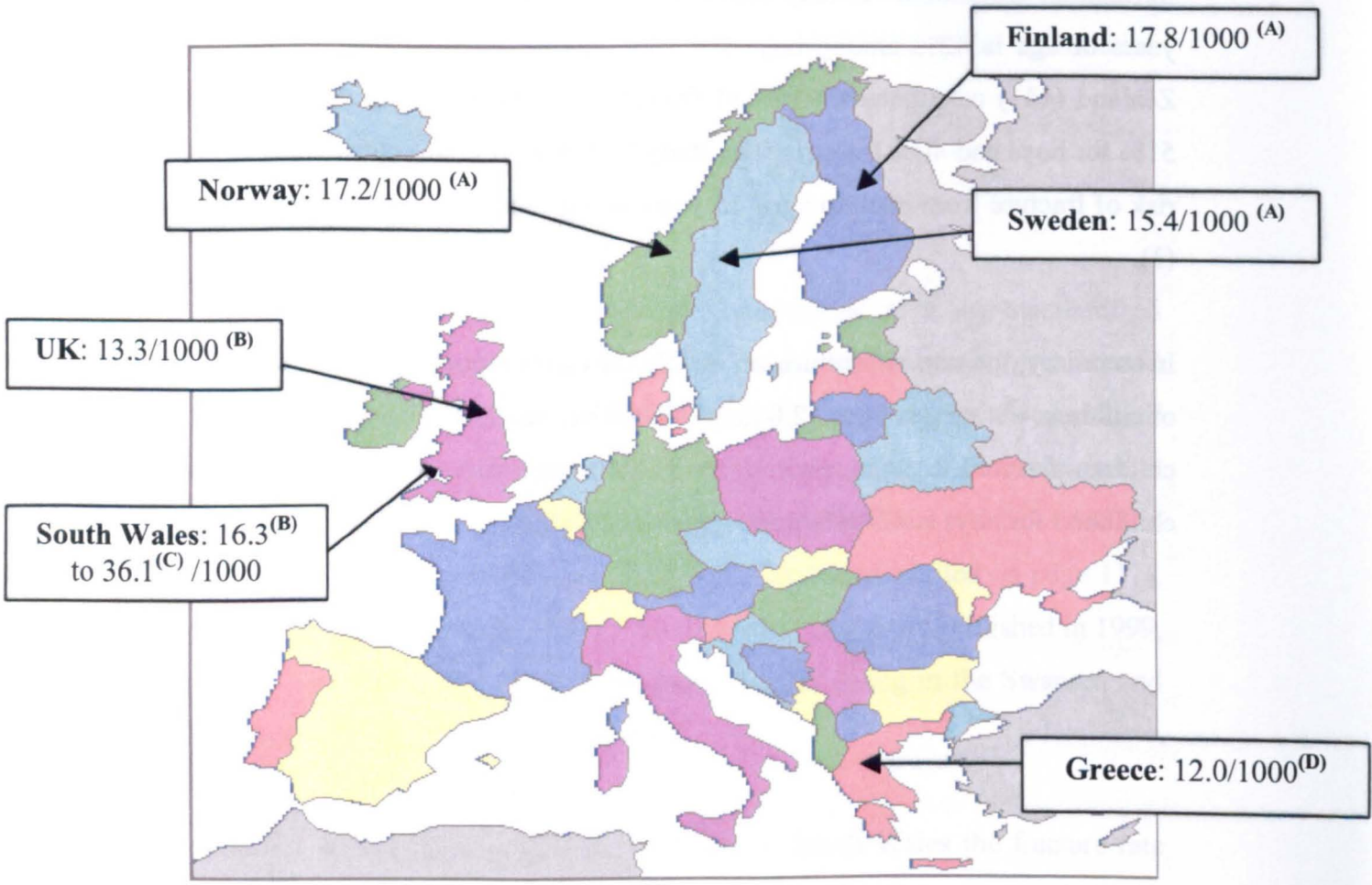
In other parts of Europe, the incidence rate of fractures per 1000 children aged 0-14 has been estimated as 12.0 in Greece (4), 15.4 in Sweden (1), 17.2 in Norway (1) and 17.8

in Finland (1). Rates of fractures have also been estimated in Norway for children aged 0 to 12 years as 12.8/1000 (12). In Sweden the rates of fracture for children aged 0 to 17 years was also estimated as 21.2/1000 (7). The results of these surveys are summarised in Figure 15, page 124, and relative latitudes are shown.

Three large population-based studies have measured the risk of sustaining a fracture throughout childhood. A study based in Sweden (7) shows this risk from birth to 16 years of age is 42% among boys and 27% among girls, whereas a study from New Zealand (431) calculated the risk of fracture from birth to aged 18 years was around 51% for boys and 40% for girls. The study by Lyons et al in South Wales calculated the risk of fracture from birth to aged 15 years was around 64% for boys and 40% for girls (3).

In summary, the rate of fractures in childhood varies according to area of study and age of children, but ranges from 12.0 to 36.1/1000 per year. In other words 1.2% to 3.6% of children fracture a bone each year. The lifetime risk of sustaining a fracture in childhood for boys is 42-64% and for girls is 27-40%.

Figure 15: Map of Europe showing incidence rates of childhood fracture.



(A) for children aged 0-14 years, Lyons et al (1)

(B) for children aged < 18 years, Cooper et al (2)

(C) for children aged 0-14 years, Lyons et al (3)

(D) for children aged 0-14 years, Moustaki et al (4)

5.1.2. Site of fracture in the skeleton

The most common fracture in children is fracture of the distal radius which accounts for 25% (7) to 43% (4) of all fractures, followed by fractures of the fingers and carpal bones (1-3,12,431). This has been detailed on page 114. Fractures of the upper limb account for 65% of all fractures (12). There does appear to be a difference in gender distribution of skeletal site of fracture: boys are more likely to have fractures of wrist/forearm or finger/hand; whereas girls are more likely to fracture wrist/forearm, finger/hand or toe/foot (431). The least common site of fracture in children aged less than 18 years is pelvis (2).

5.1.3. Repeat fractures

From 1.9 (1) to 2.7% (3,4) of children experience more than one fracture during their childhood. Having a previous history of fracture, particularly in early childhood doubles the risk of further fractures (7).

5.1.4. Age

The peak age of incidence of all childhood fractures is fairly consistent across the literature with a peak at around aged 14 years in boys, and around aged 11 years in girls, with a sharp decline in rate afterwards (2,7,431). See Figure 16, page 126.

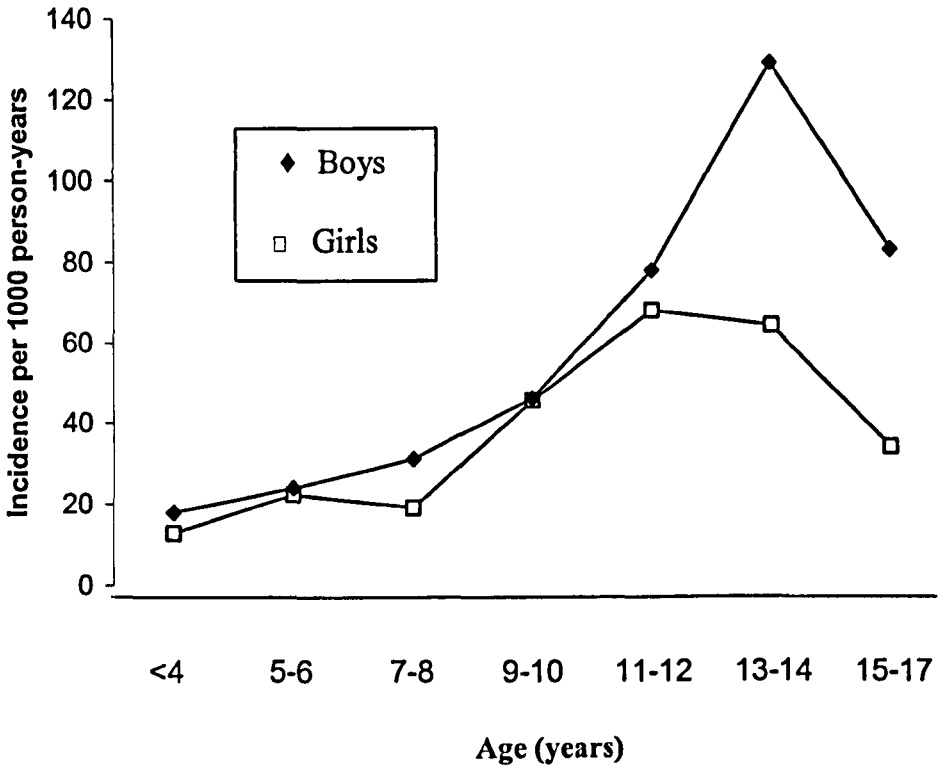
Similar age distributions are seen for distal (8,432) and proximal (433) forearm fractures. However, fractures of the femur appear to peak at around aged 2-3 years (434) and then at around aged 17 years (435). Fractures of the elbow also appear to have a different age distribution with peaks at aged 4-6 years (436).

5.1.5. Gender

Overall, boys consistently have a higher fracture rate compared with girls, at all ages. However, different skeletal sites may have different gender distributions. For elbow fractures, the rate of girls and boys is approximately equal (433,436); for distal forearm

fractures, girls have a similar or higher rate than boys before aged 9-10, but boys have a higher rate after aged 10 years (8,432).

Figure 16: The incidence of fractures per 1000 person years at various ages in childhood. Adapted from Jones et al (431).



5.1.6. Ethnicity

I found two papers describing ethnic differences in fracture rates amongst children. The first study was carried out in Baltimore, USA and looked at femoral fractures (435). The investigators observed that black children had a higher relative risk of fracture than white children (RR 1.30, 95%CI 1.17 to 1.45). This higher rate of fractures among black children was driven by the disproportionately higher relative risk for black males between the ages of 4 and 17 years. Black female children had a similar relative risk of fracture compared with their white counterparts (RR 0.87, 95%CI 0.44 to 1.32). However, the second study carried out in the UK (183) reported that ethnic minority status was a protective factor for fractures (OR 0.5, 95% CI 0.4 to 0.6, P<0.001). White

children appeared to have a similar rate of fractures to children of Pakistani origin (16.4% versus 11.2%, P=0.2), but higher rates than black children (8.8%, P=0.01) and Indian children (5.7%, P=0.002).

5.1.7. Secular trends in fractures in childhood

The population-based studies looking at all fractures over time have generally shown an increase in the rate of childhood fractures. In the study by Landin, the fracture rate in the 1970's was almost double that in the 1950's (7). For distal forearm fractures there appears to have been a more modest increase in incidence in childhood. The Dutch study by Oskam *et al* (437) showed that the incidence rate of this specific fracture increased in children aged 0-9 years from 7.5/1000 in 1971-1975 to 8.7/1000 in 1991-1995. However, the rate of fracture in children aged 10-19 years remained static. The population-based study in Rochester, USA by Khosla *et al* (8) showed that the rate of distal forearm fractures in children aged 0-4 years in 1969-1971 was 0.77/1000 but in 1999-2001 was 2.23/1000. Similarly for children aged 5-9 the rate increased from 5.3/1000 to 6.0/1000, and for children aged 10-14 years the rate increased from 8.2/1000 to 13.2/1000. Conversely, for femoral fractures (434) and tibial shaft fractures (438), the incidence appears to have been decreasing over time.

5.1.8. Management of fractures in childhood

There is very little literature on the epidemiology of the management of common childhood fractures. No studies were found from the UK, and none have been published within the last five years. One study was found which looked at distal forearm fractures in people of all ages during 1971 to 1995 that presented to a Dutch university hospital (437). This epidemiological study reports that during this time-period, hospital admissions for distal forearm fracture increased. This was largely attributable to changes in surgical policy such that there are now more indications to operate on distal forearm fractures. These include the increasing use of a general anaesthetic rather than local anaesthesia for fracture reductions, particularly in children. A few reports look at the management of rare fractures such as elbow fractures (433,439) or open forearm fractures (440).

5.1.9. Outcome of fractures in childhood

It is assumed that the majority of children with fractures have a good outcome such that after the fracture has healed there are no long-term adverse effects, although there is little empirical evidence to support this claim. The impact of childhood fractures can be divided into short-term or transient adverse effects and those that are more permanent or long-term.

5.1.9.1. Short-term adverse effects of childhood fractures

Apart from the pain associated with fractures which is difficult to quantify, short-term effects of childhood fractures include restriction of normal activity and time off school. One population based study assessed the number of activity restricted days associated with different types of childhood fractures from a county in Norway (12). The mean activity restricted days for leg fractures were 26 (95%CI 7 to 45), for arm fractures was 14 (95%CI 8 to 20) and for other fractures was 5 (95%CI 1 to 8).

In terms of time off school, there is no reason from the medical standpoint to keep a child out of action once the fracture has been immobilised and the area protected by a cast. One study assessed the advice given to parents by schools from the Bradford area of the UK (441), given three hypothetical situations: a child with an above elbow cast; a child with a below knee cast and a child with an above knee cast. Advice varied, with 17% of schools suggesting a modified activity restricted timetable for the child with the above elbow cast, 13% for the child with a below knee cast, and 30% of schools suggesting the child with the above knee cast should not attend school for a time varying from two weeks up to removal of the cast.

Other short-term effects of childhood fractures can include acute compartment syndrome in approximately 1.1% of fractures (11).

5.1.9.2. Long-term adverse effects of childhood fractures

One study was found that suggests that long-term adverse effects occur in up to 50% of ankle fractures, 43% of elbow fractures and 33% of leg/foot fractures that were described as uncomplicated fractures i.e. not displaced (442). In this study these sequelae were classified as either objective (limited joint mobility, pain on pressure,

axial deviations, weakness, or shortening of a limb) or subjective (pain at rest or during exercise, sense of unsteadiness, or numbness). Permanent sequelae appeared to be more frequent for injuries sustained by children under 10 years of age. Other long-term complications of childhood fractures include limb overgrowth (10,443), secondary osteoarthritis (13), nerve injuries (444) and bone growth failure (417,445).

5.1.10. Summary

One to 3.5% of children fracture a bone each year, and the lifetime risk of sustaining a fracture in childhood for boys is 42-64% and for girls is 27-40%. The incidence of childhood fractures appears to be increasing over time. The most common site of fracture in children is the distal radius, with a peak at around aged 14 years for boys and aged 11 years for girls. Overall, boys have a higher fracture rate than girls.

5.2. ANALYTICAL EPIDEMIOLOGY OF THE DETERMINANTS OF FRACTURES IN CHILDHOOD

The previous section described the descriptive epidemiology of fractures in childhood. This section focuses on the analytical epidemiology of the determinants of fractures in childhood. There are a number of personal attributes, often described as risk factors that may increase or decrease a child's fracture risk. These may, in themselves, be a result of composite genetic and environmental causes. They may be modifiable or non-modifiable. I have divided them into fixed and non-modifiable risk factors, risk factors with composite genetic and environmental causes, and modifiable risk factors. See Table 23 below. There are other arbitrary divisions that could have been used and divisions using a Haddon matrix is shown on page 131. In the literature review summary chapter (Chapter 7) I also divide these risk factors into those that are likely to act via skeletal fragility and those that are likely to act via alternative mechanisms (page 173).

Table 23: Categorisation of individual risk factors for fractures in childhood.

Non-modifiable risk factors	Risk factors with composite genetic and environmental causes	Potentially modifiable risk factors
Age	Bone mass	Diet and nutrition
Gender	Pubertal stage	Physical activity
Genetic determinants	Obesity	Drug treatments
Time of year	Psychological attributes	
	Balance	

There are also other factors that are related to the family environment, (see Table 24, page 131) and risk factors relating to the mechanism of injury (see Table 25, page 131). Although information is available on the impact of these individual risk factors or determinants on fracture risk in children, none of them act in isolation.

Table 24: Family risk factors for fractures in childhood.

Family risk factors
Socio-economic status
Family demographics
Home safety and environment
Family functioning

Table 25: Mechanistic risk factors for fractures in childhood.

Mechanistic risk factors
Trauma level
Landing surface
Injury type

The potential risk factors for fractures in childhood have also been summarised in a Haddon matrix:

Table 26: Haddon matrix summarising potential risk factors for fractures in childhood

	Human Factor (Host)	Agent/vehicle	Physical & Social environment
Pre-event (contributing to the probability that an injury producing event will occur in the first place)	•Age •Gender •Time of year •Psychological attributes •Physical activity •Drug treatments		•Home safety and environment •Family demographics •Family functioning •Socio-economic status
Event (contributing to the likelihood that a fracture will occur given the injury)	•Genetic determinants •Bone density / fragility •Structural properties of bones •Pubertal stage •Diet and nutrition •Drug treatments •Obesity	•Trauma level •Landing surface •Injury type	
Post-event (influencing the impact of the fracture on the child's life)	•Age •Psychological attributes •Diet and nutrition		

5.2.1. Individual non-modifiable risk factors for fractures in childhood

5.2.1.1. Genetic determinants

In a similar manner to that of bone mass discussed on page 61, it is likely that the genetic component of fracture risk is a combination of polygenic effects, gene-gene interactions and gene-environment interactions. It is therefore unlikely that a single genetic locus will by itself be the major determinant of fracture risk. However, I found one study that investigated the association between a specific gene or locus and fracture risk in children (21). In this study carried out in prepubertal Finnish girls, Suuriniemi *et al* examined the relationship between a single nucleotide polymorphism (A→C) in codon 392 in the COL1A2 gene, and retrospective occurrence of fracture. The COL1A2 gene codes for the alpha2-polypeptide of Type I collagen, the major protein constituent of bone. The polymorphism studied was at the *Pvu*II site. They found a difference in the distribution of fractures according to genotype groups (P=0.023). Children with a heterozygote genotype for the polymorphism had a risk ratio for fracture of 3.9 (1.3-11.9) while children with a homozygote genotype for the polymorphism had a risk ratio for fracture of 5.1 (1.4-18.5). However, the results of this study have not been replicated (i.e. I have found no other studies which have looked at this association), and it was based on a sample of only 244 children originally recruited for an interventional study.

5.2.1.2. Season

There is a consistent variation in childhood fracture rate throughout the seasons, and this remains after adjusting for secular trend (7). Most studies show a peak in fractures during the summer and a much lower rate during the winter (2,7,434,436,446). None of these studies adjusted for, or took account of differences in physical activity that occur throughout the year.

5.2.2. Individual risk factors with composite genetic and environmental causes for fractures in childhood

5.2.2.1. Bone mass as a risk factor for fractures in childhood

The association between estimated volumetric bone density and fractures in childhood is covered by the formal systematic review in the next chapter on page 147. In summary the results suggest that bone mass may contribute to fracture risk in childhood. This section will focus on the association between bone size and fracture risk in childhood, although some of this has been mentioned in the section on the structural geometry of bone on page 37.

Only a few studies have investigated the association between bone size and fracture risk in children. A summary is shown in Table 27 on page 134. The literature is small and results appear to depend on which bone is studied. On sub-group analysis all of the five studies report reduced size of some specific bones in children with fractures (19,22,65,447,448), but also some sub-analyses of different bones show no association. No study shows larger bone size in children with fractures.

5.2.2.2. Pubertal stage as a risk factor for fractures in childhood

The incidence of distal forearm fractures in children peaks around the time of the pubertal growth spurt (8,432,433), but whether this is due to the endocrine and metabolic changes that occur during puberty, or due to the associated changes in body structure, physical activity, diet or bio-psychosocial make-up is unknown. It has been suggested that one reason for the high fracture rate seen around the time of the pubertal growth spurt, is a lag in cortical thickness and mineralisation relative to linear skeletal growth (191). Two studies were found that investigated the association between stage of pubertal development and fracture rate (449,450). The first study was a case control study carried out in Greece on 100 children aged 7-14 years (449). Cases were defined as having a single fracture of either the upper or lower limbs. Controls were selected from outpatient clinics of the same hospital during the same period as cases, matched for age and gender. Pubertal stage was assessed by Tanner staging (as previously described on page 67). Children with fractures were much more likely to be in a higher

Table 27: Summary of the literature on the association between bone size and fracture risk in children

Author, year of publication	Age of participants	No in study	Study design	Fracture type and method of verification	Measure of bone size used	Result
Koo et al, 1988, USA (447)	preterm children at aged 1 year	74	Case control study comparing 23 infants with fractures or rickets with 51 without	Any fracture or rickets. Verified by standardised radiographs of forearms to look for fractures or rickets, and radiographs of other potential fractures	width of distal radius by single energy X-ray absorptiometry	At aged 1 year, premature children with radiographic fractures or rickets have reduced bone width of distal radius than those without (6.1mm ± 0.1 vs 6.8 ± 0.1, P<0.01)
Skaggs et al, 2001, USA (19)	4-15 year old girls	100	Case control study	Low-energy fracture of distal forearm. Unknown how/if verified	CSA and length of radius by pQCT, adjusted for height and weight.	No association with radial length. CSA adjusted for height and weight smaller in girls with fractures compared to those without (1.83 cm^2 ± 0.34 versus 1.99 ± 0.33, P=0.0001)
Goulding et al, 2001, New Zealand (65)	3-19 year old boys	200	Case control study	Distal forearm fractures verified by X-ray.	TB bone area by DXA	TB bone area smaller in children with fractures compared to those without (1722 cm^2 ± 556 versus 1743 ± 574, P<0.05)
Ma and Jones, 2003, Tasmania (22)	9-16 years	642	Case control study	Upper limb fractures. Unknown how/if verified	Outer width, inner width and cortical width of metacarpal bone by radiogrammetry	No difference in outer width, but greater inner width and smaller cortical width in children with fractures compared to those without. OR for subgroup of wrist and forearm fractures per SD decrease in cortical width of 1.6 (95%CI 1.2 to 1.9, P<0.01)
Goulding et al, 2005, New Zealand (448)	5-19 years	90	Cross-sectional study comparing children with multiple fractures to fracture-free reference populations studied in the same research unit	Repeated forearm fractures. Unknown how/if verified	Area and width of ultra-distal radius, one-third radius and lumbar spine by DXA. Area of femoral neck, hip trochanter and total body by DXA.	No association with area of ultra-distal radius, one-third radius, femoral neck, hip trochanter or total body. No association with width of ultra-distal radius, but reduced bone width of one-third radius and L spine in children with fractures compared to reference populations, P<0.05

Abbreviations: CSA cross sectional area; DXA dual energy X-ray absorptiometry; pQCT peripheral quantitative computed tomography; SD standard deviation; TB total body; USA United States of America

Tanner Stage than those without fractures: odds ratio (OR) for Tanner stage 1 was taken as baseline (1.0), for Tanner stage 2 was 1.7 and Tanner stage 3 or above was 3.4. 95%CI were not given in the paper, but the P value for the test for trend was 0.01. The second study (450) used the GOOD cohort (Gothenburg Osteoporosis and Obesity Determinants study) which consists of 1068 young men aged 18.9 years ± 0.6 randomly chosen from the greater Gothenburg area of Sweden. In this particular study a subset of 642 subjects had complete growth charts available for determination of peak height velocity prior to recruitment for the study. Data were also collected on fractures occurring prior to enrolment, but age at fracture was not recorded. Results showed that age at peak height velocity, which occurs within two years of the onset of puberty in boys, was an independent predictor of previous upper limb fractures, with an odds ratio for fracture risk of 1.35 (95%CI 1.04 to 1.75) per year increment in age at peak height velocity.

Three other studies mention in their results section that no association was found between pubertal stage and fracture rate, but no actual values for the association were presented (18,65,451). These results are surprising given the strong age pattern of fractures. However, the association between puberty and fractures was not the main focus of any of these papers, and the three null studies were small and therefore insufficiently powered to detect important associations.

5.2.2.3. Obesity as a risk factor for fractures in childhood

In adults it is well-recognised that obesity is protective for postmenopausal bone fractures (452). However, in children, there is contradictory evidence for the association between obesity and fracture risk. See Table 28, page 136 for a summary of the studies. No association between body weight and fracture risk was reported in 4 case control studies (18,20,24,453); one cross-sectional study reported girls with fractures were more overweight than average (19); one case control study reported boys with fractures were more overweight than those without (65); and one prospective cohort study reported a positive association between weight and prepubertal fractures but not peri- or post-pubertal fractures (454). Similar contradictory results were seen for BMI, fat mass measured by DXA, percentage fat or waist circumference. Therefore no conclusions can be drawn from the current evidence.

Table 28: Summary of the studies investigating the association between obesity and fracture risk in children

Author, year of publication	Age of participants	No in study	Study design	Measure of obesity	Result
Jones et al, 2003, New Zealand (454)	< 18 years	968	Prospective cohort study	weight, BMI	Positive association between weight and BMI with prepubertal fractures, not adolescent fractures. OR per SD increase in weight from aged 5 to 18 years = 1.37, 95%CI 1.12 to 1.67; OR per SD increase in BMI = 1.24, 95%CI 1.02 to 1.52.
Goulding et al, 2004, New Zealand (24)	3-13 years	50	Case control study: all children enrolled avoided cow's milk. Cases had fractures, controls did not	weight, BMI, overweight defined as BMI above 85th percentile for age	No difference in weight, BMI or percentage overweight in those with and without fractures
Goulding et al, 2003, New Zealand (453)	10-21 year old boys	93	Case control study: cases had distal forearm fractures, controls did not	weight, BMI, fat mass, % fat, waist circumference	No association found for any measure of obesity and fracture risk, P>0.05 for all
Ma & Jones, 2002, Australia (20)	8 years	324	Nested case control study from a cohort set up to study the long-term effects of being at an increased risk of Sudden Infant Death Syndrome.	weight	No association - actual results not presented in paper
Goulding et al, 2001, New Zealand (65)	3-19 year old boys	200	Case control study: cases were boys with distal forearm fracture confirmed by X-ray films, living in a defined area. Controls were friends of cases	weight, BMI, % fat and fat mass by DXA	More children with fractures were overweight (36 vs 14, P<0.001). Odds ratios for those with a Z score of <-1 compared with >+1 for weight was 2.07 (95%CI 1.03 to 4.15), for fat mass was 3.19 (95%CI 1.55 to 6.56) and for BMI was 3.47 (95%CI 1.69 to 7.09).
Skaggs et al, 2001, USA (19)	4-15 year old girls	100	Cross-sectional analysis: compared body weight in girls with fractures to age-adjusted normal percentiles for growth. Case control study: cases were girls with low-energy forearm fractures. Controls were healthy volunteers from schools. Forearm fat area used	body weight and forearm fat area measured by pQCT	Mean weight for girls with fractures was at 90th percentile for mean age-adjusted values for normal girls. No association between forearm fat area and fracture risk
Goulding et al, 1998, New Zealand (18)	3-15 year old girls	200	Case control study: cases were Caucasian girls with distal forearm fractures confirmed by X-ray. Controls were friends of cases	weight, fat mass by DXA	No association with weight. Positive association with fat mass: OR for fracture risk for those with a fat mass Z score of < -1 compared with > +1 of 2.39 (95%CI 1.82 to 4.83)

Abbreviations: BMI body mass index; CI confidence interval; DXA dual energy X-ray absorptiometry; OR odds ratio; pQCT peripheral quantitative computed tomography; SD standard deviation; USA United States of America

5.2.2.4. Psychological status as a risk factor for fractures in childhood

Attention Deficit Hyperactivity Disorder (ADHD) is an externalising disorder characterised by developmentally inappropriate levels of inattention (difficulty in concentrating), hyperactivity (disorganised, excessive levels of activity) and impulsive behaviour (455). All these components of behaviour may increase either the opportunity for injury, or the likelihood of fracture should an injury occur. A study looking at children from England, Scotland and Wales (456) found that children with ADHD had an increased risk of fractures (OR 1.6, 95% CI 1.2-2.3, adjusted for age and gender) compared to normal controls.

The same study looking at children from England, Scotland and Wales (456) used the Development and Well-Being Assessment (DAWBA) interview to make a range of psychiatric diagnoses as described in the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV). They also found that children with depression had an increased risk of fractures (OR 1.9, 95% CI 1.1-2.3, adjusted for age and gender) compared to normal controls.

Risk-taking behaviour can be defined as acting without fear of the consequences on health, emotions, life or the future (457). It is a normal component of adolescent bio-psychosocial development, but may increase fracture risk by increased engagement in physically daring activities. A study based in Australia (458) measured risk-taking behaviour in children and found this was associated with a higher risk of hand fractures (unadjusted OR 2.55, 95%CI 1.27, 5.11), but not fractures at other sites.

Children with behavioural difficulties or conduct problems, as defined in DSM-IV, may be more likely to disregard rules such as those related to safety, and be more likely to fight with other children. Poorer school behaviour has been shown to be more common in children with fractures (459).

5.2.2.5. Balance as a risk factor for fractures in childhood

Adults with poor balance and high postural sway fall more often and have a higher bone fracture risk than adults with better postural control (460). One study has assessed

balance and the risk of fracture in children (453), and in this, no association was seen between adverse balance performance and fracture rate. However, the overweight boys had poorer balance than those of healthy weight. No evidence was found that children with poorer balance are risk averse.

5.2.3. Potentially modifiable individual risk factors for fractures in childhood

5.2.3.1. Diet and nutrition

Studies have been found that investigated the association between calcium intake, carbonated beverage intake and total energy intake and fracture risk in children, and are summarised in Table 29 on page 139. Adequate absorption of calcium intake is important for optimal bone growth (461). Two studies reported an inverse association between calcium intake and fracture risk in children (462,463), but three found no association (24,449,464). The methods of measuring calcium intake varied between studies, and total energy intake was not assessed or adjusted for in four of the studies.

A positive relationship between cola beverage intake and fracture risk in children has been reported in three studies: one reported an association with any upper or lower limb fracture (449); one for wrist and forearm fractures only, not all upper limb fractures (464); and one in girls only, not boys (463). Whether this association is due to a potential effect of excess phosphorus in the cola impairing bone mineral status, or due to a lack of calcium in the diet because of substitution of cola for dairy products is unclear (465). However, the study by Ma and Jones (464) showed that the association between cola drink consumption and fracture risk in children may be mediated by increased television viewing (i.e. decreased physical activity), and not by decreased milk intake. This implies that cola drink consumption is a behavioural marker, rather than a causal factor *per se*.

Table 29: Summary of studies investigating the association between diet or nutrition and fracture risk in children

Author, year of publication	Age	No in study	Study design	Measure of calcium intake	Measure of carbonated beverage intake	Measure of total caloric intake	Result
Ma & Jones, 2004, Tasmania (464)	9-16 years	412	Case control study of children with and without upper limb fractures	Face-to-face interview to calculate frequency of dairy drink (any milk drink) intake in one week. Calcium intake from other sources not assessed.	Face-to-face interview to calculate frequency of cola intake in one week. Frequency of other non-cola carbonated beverages was also determined	Not assessed	No association between any drink intake and total upper limb fractures. Limiting to wrist and forearm fractures, there was a positive association between cola drink consumption and fracture risk of OR 1.39 per 300ml increase in cola intake per week (approximately one drink), 95%CI 1.01 to 1.91.
Goulding et al, 2004, Australia (24)	3-13 years	1050	Cross-sectional design comparing 50 children with cow's milk avoidance with 1000 children from a birth cohort in the same city	'current dietary calcium intake was estimated'		Not assessed	No association between calcium intake and fracture risk: 27% of fractures had calcium intake below 300mg per day compared with 31% of controls
Chlebna-Sokol et al, 2003, Poland (462)	9-17 years	74	Case control study of children with skeletal abnormalities including fractures compared with healthy children	3-day diet interview performed with a computer programme FOOD3.1		unable to extract enough data from abstract	Higher incidence of low calcium intake in children with skeletal abnormalities including fractures compared with normal controls (actual results not presented in abstract)
Petridou et al, 1997, Greece (449)	7-14 years	200	Case control study of children with single fractures of upper or lower limb compared with healthy controls	semi-quantitative food frequency questionnaire focusing on dairy products	semi-quantitative food frequency questionnaire focusing on non-alcoholic beverages	Not assessed	No association between intake of dairy products or non-cola carbonated beverages and fractures. Positive association between cola-beverages and fracture risk of OR 1.7 per increase in half a can of drink per day, 95%CI 1.2 to 2.6
Wyshak et al, 1994, UK (463)	15 years	127	Case control study of children with and without fractures	food frequency questionnaires	food frequency questionnaires	food frequency questionnaires	Inverse association between calcium intake and fracture risk of OR 0.28, 95%CI 0.09 to 0.92 No association between non-cola carbonated beverages and fracture risk Positive association between cola beverage intake and fracture risk in girls OR 3.59, 95%CI 1.21 to 10.75. No association in boys Inverse association between total caloric intake and fracture risk in boys only. No association in girls (actual results not given in abstract)

Abbreviations: CI confidence interval; OR odds ratio; UK United Kingdom

5.2.3.2. Physical activity

Two studies on the same cohort of children from Tasmania (464,466) have shown that time spent watching television or videos, or playing on a computer was associated with increased upper limb fractures in both boys and girls (OR 1.6, 95%CI 1.1 to 2.2). The implication of this is that less time is spent in physical activity, although a recent meta-analysis (467) suggests that the relationship between TV viewing and physical activity is negative but small, and watching television may replace other sedentary activities such as reading, rather than replacing physical activity. One of the studies investigating the association between physical activity and fractures in children (466) also showed that involvement in light physical activity decreased fracture risk (OR 0.8, 95%CI 0.7-1.0), but that sports participation increased hand and upper arm fractures in boys whilst decreasing these in girls. Another study by the same authors (20), showed that children with higher levels of sports participation had an increased risk of fractures. In these studies the measures of physical activity are poor and are measures of either sedentariness or sports participation.

A study of children in north east England (468) looked specifically at fractures occurring during sporting activities, and found that 20% of sporting-related injuries resulted in a broken bone. Overall, football, rollerblading, cycling and netball injuries were the commonest cause of fractures. Among the boys, football and rollerblading injuries, and among the girls rollerblading and netball injuries were the commonest cause of fractures. It is likely that these are the most frequent sports played by children, and no attempt had been made to adjust for time spent in different activities. The fracture risk for a particular sport depends on the frequency that the sport is played, and the risk associated with taking part in the sport.

Other studies on the epidemiology of fractures suggest that 36% (3) to 50% (436) of fractures in childhood are related to sporting activities. The specific types of sporting activity seems to vary by country. For example, winter-sports such as sledding, ice-skating and skiing seem to be particularly associated with fractures in Denmark (432), while team sports with a ball or wheel seem to be more associated with fractures in the UK (3).

5.2.3.3. Drug treatments

Oral corticosteroids are well-known to increase the risk of fracture in adults (469). An observational study has looked at the use of oral corticosteroids and fracture risk in children (348). It found that children who received four or more courses of oral corticosteroids per year had an increased risk of fracture (OR 1.32, 95%CI 1.03, 1.69). Fracture risk was also increased in children using 30mg prednisolone per day (OR 1.24, 95%CI 1.00, 1.52). It is not entirely clear if this increased risk relates to the direct effects of oral corticosteroids, or to the underlying disease and its severity.

Two studies have also looked at the association between inhaled steroids and fracture risk in children (349,470). They both used the UK GP Research Database and reported similar results. The study by Schlienger *et al* (470) identified 3,744 cases of fracture in children aged 5 to 17 years and 21,757 matched controls. They found that current exposure to inhaled steroids was not associated with a substantially altered fracture risk. The study by van Staa *et al* (349) identified 23,984 non-vertebral fractures in children aged 4 to 17 years with an equal number of matched controls. They found a dose-dependent relationship between inhaled beclomethasone dose and fracture risk (OR 1.36, 95%CI 1.11 to 1.67 for daily doses over 400mcg). This excess risk disappeared after adjusting for indicators of asthma severity, suggesting it may be due to the underlying illness rather than directly attributable to inhaled corticosteroid therapy.

5.2.4. Family risk factors for fractures in childhood

5.2.4.1. Socio-economic status

Two population-based studies have investigated the association between socio-economic status and fractures in childhood, but reported contradictory results. The first study based in Glasgow (5) assessed socio-economic status by neighbourhood-type analysis and found that children aged 0 to 14 years living in deprived areas had higher fracture rates than those from affluent areas (fracture rate 175.3/10 000 children in deprived areas, 140.1/10 000 in affluent areas, P value for comparison < 0.001). However, the similar study based in South Wales (6) which used Townsend scores to assess socio-economic status found no association between deprivation and childhood fracture rates.

5.2.4.2. Family demographics

I found two studies that assessed the effects of family demographics on fracture risk in children. In the first study by Loder *et al*, which described the psychosocial characteristics of children with fractures without comparison to a control group (459), children with fractures came from families with an average number of children per family of 2.6 ± 1.1, range 1 to 6; were later in the birth order (average birth rank of child with fracture was 1.9 ± 1, range 1 to 4); and there tended to be more boys in the family compared with girls (ratio of 1.25 to 1). However, in the second study (456), family size was not associated with fracture risk in children.

5.2.4.3. Home safety and environment

In the study by Loder *et al* (459), parents of children with fractures perceived the overall safety of the home as quite good. However, a study by Mott *et al* (471), showed that children living in homes described as 'dark' or 'cluttered' by an independent observer were more likely to sustain an injury.

5.2.4.4. Family functioning

The same study by Loder *et al* (459), showed that children who fractured a bone at home came from more dysfunctional families than those who fractured a bone away from home ($P<0.05$: no effect size reported in the paper). Here, family dysfunction was defined as poor problem-solving ability as a family, poor communication, lack of understanding of individuals roles within the family, depressed or neurotic affective responsiveness, depressed or neurotic affective involvement, poor behaviour control and general poor functioning of dynamics within the family.

5.2.5. Mechanistic risk factors for fractures in childhood

5.2.5.1. Trauma level

The first and largest paper on trauma level resulting in fractures in children is by Landin (7). In this seminal paper, trauma was divided into slight, moderate or severe by application of descriptions or examples of injuries by the author. See page 117 for a full description. This study showed that during the period 1975 to 1979, 66% of fractures in children aged 0 to 14 years were caused by slight trauma, 19% by moderate trauma, 7%

by severe trauma, and 8% were not classifiable. During a 30 year observation period the annual incidence of fractures caused by slight trauma increased by 30% ($P<0.001$). Fractures of the carpal-metacarpal region, hand and foot phalanges, ankle and distal forearm were commonly produced by low energy trauma. Fractures of the skull and femur were more often produced by higher energy levels. However, no data were available on the frequency of the various activities in the general population, and so no denominator was available.

There is circumstantial evidence from the injury literature to support some of the examples used by Landin to gauge trauma level in injuries that cause fractures in children. Many studies have shown that falling from greater than 3 meters increases the risk of injury (386,472-474), whereas a study of children who fell 3-4 feet whilst in hospital showed that these types of falls resulted in either no injury, or very minor injuries (474).

5.2.5.2. Landing surface

I found no studies on the association between landing surface and fractures in children. However, there are many studies assessing the effect of landing surfaces on injuries in general, usually focusing on the surface characteristics of playgrounds. In fact, Cummins and Potter (393) suggest that the contact surface is more important than the height of the fall when assessing mechanistic severity. When looking at playground surfaces, non-resilient surfaces such as concrete are more likely to result in a serious injury (386), whereas surfaces such as sand, and rubber result in less serious injury (472,475). Bark surfacing alone is better than concrete, but not as good as rubber (475). Also, bark surfacing needs to be of appropriate depth – a depth of less than 8cm is ineffective in attenuating the risk of playground falls, and does not reduce the risk of severe head injury (476).

5.2.5.3. Injury type

Falls are the cause of around half of all distal forearm fractures (7,8) and femoral fractures (434) in childhood. In the later study, falls were recorded as the cause of femoral fractures in 76.7% of 1 year old children and 26.2% of 8 year old children.

Between 1975 and 1979 in Sweden, Landin (7) found 12% of fractures were due to Road Traffic Injuries (RTIs), defined as either a pedestrian hit by a vehicle, a car passenger, or involving a bicycle, moped or motorcycle. This study also showed that RTIs causing fractures increased over 30 years by a third.

However, a later study in West Midlands, UK (434) showed femoral fractures due to RTIs fell by 43% per year over the period 1991 to 2001. In fact, RTIs were the external cause of femoral fractures in 2.2% of 1-year-old children and 54.0% of 10-year-old children (overall 28.0% of children aged under 16). A study carried out in various European districts found RTIs were responsible for just 1.4% of all fractures (1).

In the study from Sweden (7), fractures due to fights increased over the 30 year period, particularly in young teenage boys. More than 90% of childhood fractures sustained during fighting were localised to the hand and facial skeleton.

5.3. SUMMARY OF DETERMINANTS OF FRACTURES IN CHILDHOOD

- Fractures in children are common: the incidence ranges from 1% to 3.6% per year, and the rate seems to be increasing

- Boys more commonly fracture than girls, and the most common site of fractures is the distal forearm

- There is a consistent association between cola beverage intake and fracture risk in childhood, although it is more likely that cola drink consumption is a behavioural marker rather than a causal factor per se

- 36% to 50% of fractures in childhood are related to sporting activities

- 66% of childhood fractures are caused by slight trauma defined as a fall from less than half a meter, playing injuries including playground scuffles or low energy sporting injuries such as ball sports, skating, wrestling, judo, karate and gymnastics

- Falls are the cause of around half of all distal forearm fractures in childhood

CHAPTER 6: THE ASSOCIATION BETWEEN BONE MASS AND FRACTURES IN CHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS.

The previous chapters have covered the structure and properties of bone, the determinants of bone mass in childhood, childhood injury epidemiology and the determinants of fractures in childhood. This chapter is a systematic review and meta-analysis of studies available in the literature investigating the association between bone mass and fractures in children. An article has been published in a peer reviewed journal based on this systematic review, see Appendix A, page 401.

6.1. INTRODUCTION

As previously discussed on page 41, in adults, low bone mass, or osteoporosis is a risk factor for fracture (79). Despite this well-recognised association between osteoporosis and fracture risk in adults, the association between bone mass in children and fracture risk in childhood is unclear. Indirect evidence that suggests bone mass may influence fracture risk in children is available from several randomised double-blind intervention trials that examined the effects of calcium intake in children and adolescents (270,281,282). These studies demonstrated improvements in bone mass, and a further study found that children who avoid drinking cow's milk are at an increased risk of pre-pubertal bone fractures (477).

As bone mass is the main explanatory variable I am interested in, the objective of this chapter was to systematically review all published studies investigating the association between bone density and fractures in children.

6.2. METHODS

All observational epidemiological studies that examined the relationship between bone mass and fractures in children were included. Children were defined as less than or equal to 16.0 years of age. Children were excluded if they had a chronic illness likely to affect bone mass. All studies were required to have extractable data on bone mass measured by any method. The primary outcome measure was all fractures.

A systematic strategy was used to search electronic databases of published articles using both MeSH headings and text-words. The databases searched were MEDLINE (1966-2005), EMBASE (1988-2005), Web of Science (1965-2005), the Cochrane Musculoskeletal Injuries Group, the Cochrane Controlled Trials Register and SIGLE for 'grey' literature. Articles about bone mass were identified by using the words "bone density", "bone mineral density", "bone mineral content", "bone mass", "bone mineral apparent density" or "calcification" and their abbreviations. Articles about fractures were identified using the words "fracture" or "fractures". Articles on children were identified by either using MeSH headings of "infant", "child" or "adolescent", or by limiting the search. Reference lists of articles obtained were also searched.

I assessed the methodological quality of the studies. If the article did not contain sufficient information on the methodology, the authors were contacted. The key components of study quality assessed were comparability of fracture and control group at entry; selection of control group; definition of inclusion and exclusion criteria; clearly defined outcome measures; the measure and control for potential confounders in either the recruitment or analysis stage; and use of multiple comparisons or subgroup analyses.

The methods and results of all studies reporting the association between bone density and fracture risk in children were tabulated. Data from studies reporting means and standard deviations were combined. A test of heterogeneity was performed and a Funnel Plot drawn to look for asymmetry due to publication bias and heterogeneity (478). Analysis was performed using STATA version 8.0 using the 'metan' and 'funnel' commands. The Standardised Mean Difference (SMD) was calculated by the difference

in means divided by the pooled standard deviation of participants' outcomes across the whole trial (479).

6.3. RESULTS

Two hundred and fifty seven articles were identified with the search strategy. Two hundred and thirty four were rejected after reading the title and abstract because they included children older than 16 years, included children with chronic illnesses, were not relevant, were case reports, had no measure of association, were letters that did not report original research findings, were duplicate references or had no fractures. Twenty three full papers were retrieved. Of these, thirteen were rejected. See Table 26, page 150 for a description of these thirteen rejected studies. Ten case control studies were identified and included in this review (15-24). There were no prospective studies found. A flow chart summarising this process is shown in Figure 17 on page 151.

The methods of the 10 case-control studies investigating the association between bone density and fracture risk in children are described below and summarised in Table 27, page 161.

Six studies showed an association between low bone mass and fractures in children (15,16,18,21-23). Four studies showed no association (17,19,20,24). The number of cases (fractures) in each study ranged from 16 (24) to 321 (22). Three studies recruited only females (18,19,21), but the rest recruited both sexes. It was unclear how fractures were verified in five studies (15-17,19,23). Two studies (23,24) did not control for potential confounders, either during recruitment or analysis. All other studies controlled for the potential confounding effects of age; six studies controlled for the potential confounding effects of body size (15,19-22,24). Only one study (24) explicitly stated that the assessor of bone density was blinded to the child's case or control status. Dual energy X-ray absorptiometry (DXA) alone was used to assess bone density in eight studies; one study used computed tomography (CT) (19); one study used qualitative ultrasound (QUS) (23); and one study used all three methods (21).

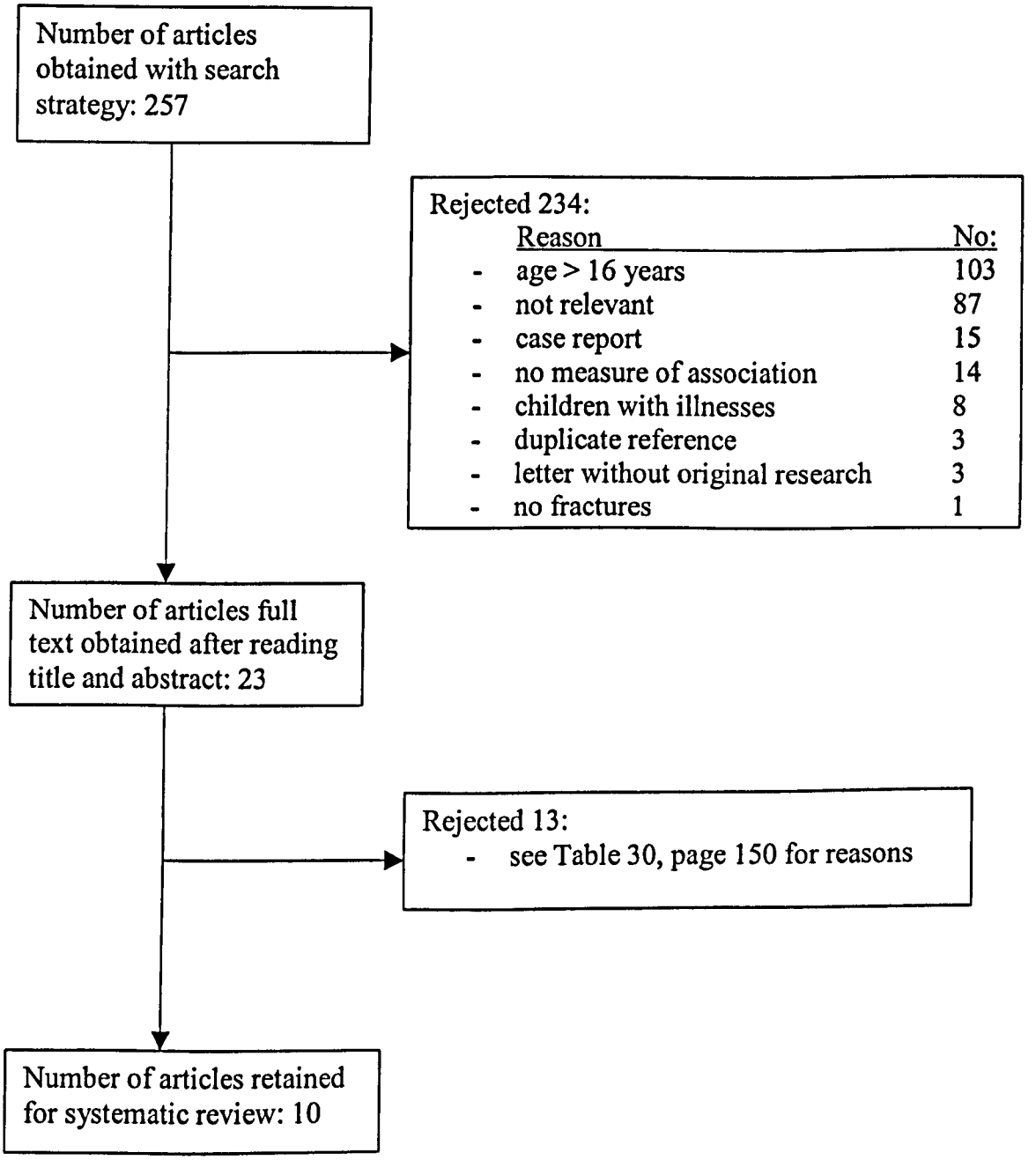
Table 30: Rejected articles for this systematic review of the association between bone mass and fracture risk in children

Article	Reason for Rejection *
Elsasser et al, Bone rarefaction and crush fractures in JCA. Arch Dis Child 1982; 57: 377-380 (480)	Unknown age of children, chronic disease (juvenile arthritis)
Koo et al, Sequential BMC in small preterm infants with and without fractures and rickets. J Bone Miner Res 1988; 3: 193-197 (447)	Unable to obtain data for children with and without fractures
Hagino et al, Fracture incidence and BMD of the distal radius in Japanese children. Arch Orthop Trauma Surg 1990; 109: 262-264 (481)	No bone density measurements
Blimkie et al, Fractures, physical activity and growth velocity in adolescent Belgium boys. Med Sci Sports Exerc 1992; 25: 801-808 (191)	No bone density measurements
Greenfield D. Risk factors for fracture [PhD thesis]. Sheffield, UK: Sheffield University; 1998.	Adults
Goulding et al, More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 2000; 15: 2011-2018 (451)	Identical study children as in ref (18)
Goulding et al, BMD and body composition in boys with distal forearm fractures: a DXA study. J Pediatr 2001; 139: 509-515 (65)	Included children > 16 years of age
Jones et al, How many children remain fracture free during growth? A longitudinal study of children and adolescents participating in the Dunedin Multidisciplinary Health and Development Study. Osteoporos Int 2002; 13: 990-995 (431)	No bone density measurements
Jones et al, Four year gain in bone mass in girls with and without past forearm fractures. J Bone Miner Res 2002; 17: 1065-1072 (482)	Identical study children as in ref (18)
Davidson et al, Biomechanical analysis of arm fracture in obese boys. J Paediatr Child Health 2003; 39: 657-664 (483)	Included children > 16 years of age
Goulding et al, Dynamic and static tests of balance and postural sway in boys: effects of previous wrist bone fractures and high adiposity. Gait Posture 2003; 17: 136=141 (453)	Included children > 16 years of age
Ma & Jones, TV, computer and video viewing; physical activity and upper limb fracture risk in children. J Bone Miner Res 2003; 19: 1970-1977 (466)	Values of bone mass in fractures and controls not presented
Ma & Jones, Soft drink, milk consumption, physical activity, bone mass and upper limb fractures in children. Calcif Tissue Int 2004; 75: 286-291 (464)	Identical study children as in ref (20)

* Study entry and exclusion criteria for this systematic review: Children were defined as less than or equal to 16.0 years of age. Children were excluded if they had a chronic illness likely to affect bone mass. All studies were required to have extractable data on bone mass measured by any method. The primary outcome measure was all fractures.

Abbreviations: BMC bone mineral content; BMD bone mineral density; DXA dual energy X-ray absorptiometry; JCA juvenile chronic arthritis; TV television

Figure 17: Summary of search strategy and papers retrieved for this systematic review of the association between bone mass and fracture risk in children



In all studies, the measure of bone density was taken after the fracture with the time delay ranging from 12 hours (23) to more than one year (21). As all these studies were observational in nature, there is always the potential for bias and confounding, both measured and unmeasured.

6.3.1. Case Control Study 1: Landin & Nilsson, 1983 (15)

This study was carried out in Malmo, Sweden. It included 221 boys and girls aged 4-16 years. 90 children had fractures (cases) and there were 131 controls selected from the same population as the cases, although the exact method of control selection is unknown. This case control study was specifically set up in an attempt to examine skeletal BMC in children who recently sustained fractures in comparison with a control population of healthy children. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the radius within 40 days of fracture. Results showed an association between forearm BMC adjusted for age and weight in boys, and fractures caused by low-energy trauma (-7.9% difference in means, $P<0.05$). No evidence was seen for an association in girls, or for fractures caused by high- to moderate-energy trauma in either gender. Results were not given for both sexes combined. The following methodological issues were identified:

- unclear how controls were selected, but stated to be 'from the same population as the fracture patients'
- comparability of cases and controls at baseline not described
- unclear if any verification of fractures
- bone density measured approximately 40 days after the fracture had occurred
- adjusted for the potential confounding effects of age, body size by weight, height and body surface
- pubertal status not assessed

6.3.2. Case Control Study 2: Chan et al, 1984 (16)

This study was carried out in Salt Lake City, USA. It included 34 boys and girls aged 2-12 years. 17 children had fractures (cases). This case control study used 17 controls matched for age (within six months), race and gender although the exact method of

control selection is unknown. Cases were children who required hospitalisation and immobilization for a limb fracture. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the radius approximately 16 months after fracture occurred. Results showed an association between forearm BMC adjusted for age, gender and ethnicity, and fractures (mean in fractures 0.423g ± 0.042, mean in controls 0.461g ± 0.037, P<0.05). The following methodological issues were identified:

- small numbers of children
- unclear how controls were selected
- comparison of cases and controls at baseline showed a difference in weight
- no obvious verification of fractures, but all children were hospitalised and immobilised
- bone density measured approximately 16 months after the fracture had occurred
- no adjustment for the potential confounding effects of body size in analysis or recruitment phase, but adjustment for age
- pubertal status not assessed

6.3.3. Case Control Study 3: Cook et al, 1987 (17)

This study was carried out in Louisiana, USA. It included 34 boys and girls aged 3-14 years. 17 children had fractures (cases). This case control study used 17 controls matched for age, race and gender although the exact method of control selection is unknown. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the lumbar spine and femoral neck within four weeks of fracture. Results showed no association between lumbar spine BMC adjusted for age and ethnicity, and fractures (mean in fractures 21.5g ± 8.1, mean in controls 23.1g ± 10.4 P=0.77). No evidence was seen of an association between lumbar spine BMD adjusted for age and ethnicity, and fractures (mean in fractures 0.759g ± 0.131, mean in controls 0.760g ± 0.137, P=0.99). The following methodological issues were identified:

- small numbers of children
- unclear how controls were selected

- cases and controls were comparable at baseline
- no obvious verification of fractures
- bone density measured approximately 4 weeks after the fracture had occurred
- controlled for the potential confounding effects of age. No adjustment for the potential confounding effects of body size in analysis or recruitment phase, but noted that there were no differences between the two groups in terms of height or weight
- pubertal status not assessed

6.3.4. Case Control Study 4: Goulding et al, 1998 (18)

This study was carried out in Dunedin, New Zealand. It included 200 girls aged 3-15 years. 100 children had fractures (cases), and there were 100 friends of cases of the same age who acted as controls. This case control study was specifically set up to determine whether children with a recent forearm fracture have lower bone density than age-matched children who have never had a fracture. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the lumbar spine, femur, total body and radius within six weeks of fracture. The results showed an association between lumbar spine BMD adjusted for age in girls aged 11 to 15 and distal forearm fractures (mean in fractures 0.916g/cm^2 ± 0.195, mean in controls 1.001 g/cm^2 ± 0.165, P<0.05); forearm BMD adjusted for age in girls aged 11 to 15 and distal forearm fractures (mean in fractures 0.300 g/cm^2 ± 0.043, mean in controls 0.320 g/cm^2 ± 0.042, P<0.05); and forearm BMC adjusted for age in girls aged 11 to 15 and distal forearm fractures (mean in fractures 1.048 g/cm^2 ± 0.219, mean in controls 1.165 g/cm^2 ± 0.231, P<0.05). No evidence for a similar association was seen in girls aged 3 to 7 or 8 to 10. No evidence was seen for an association between total body BMC, total body BMD or neck of femur BMD and distal forearm fractures. The following methodological issues were identified:

- use of neighbourhood controls
- comparison of cases and controls at baseline showed a difference in weight
- fractures were verified by X-ray or chart review where possible, but it is not stated how many cases were verified in this way
- bone density measured approximately 6 weeks after the fracture had occurred

- no adjustment for the potential confounding effects of body size in analysis or recruitment phase, but noted that there were no differences between the two groups in terms of height
- children were assumed to be pre-pubertal because of their ages, but no formal testing of pubertal stage had been undertaken.
- multiple comparisons with increased likelihood that a 'significant' result will be seen by chance
- subgroup analyses

6.3.5. Case Control Study 5: Skaggs et al, 2001 (19)

This study was carried out in California, USA. It included 100 girls aged 6-15 years. 50 children had fractures (cases), and there were 50 community controls matched for age, Tanner stage, height and weight. This case control study was specifically set up to determine the structural basis for the skeletal fragility observed in children who sustain forearm fractures and to determine if this deficiency is caused by lower bone mineral or increased cortical porosity. Exclusion criteria are listed in Table 27 on page 161. Bone density was measured by pQCT of the radius within one month of fracture. Results showed no association between cancellous bone density adjusted for age, height, weight and puberty, and distal forearm fractures after low-energy impact trauma (mean in fractures 256g/cm^3 ± 37, mean in controls 264g/cm^3 ± 30, P=NS). No evidence was seen for an association between integral bone density adjusted for age, height, weight and puberty, and distal forearm fractures after low-energy impact trauma (mean in fractures 388g/cm^3 ± 69, mean in controls 396g/cm^3 ± 63, P=NS). No evidence was seen for an association between cortical bone density of the forearm adjusted for age, height, weight and puberty, and distal forearm fractures after low-energy impact trauma (mean in fractures 1970g/cm^3 ± 54, mean in controls 1971 g/cm^3 ± 54, P=NS). The following methodological issues were identified:

- use of neighbourhood controls
- cases and controls were comparable at baseline
- no obvious verification of fracture
- bone density measured approximately 1 month after the fracture had occurred
- adjusted for the potential confounding effects of age and body size by height and weight in the recruitment phase

- puberty was assessed by Tanner stage, and cases and controls were matched for this

6.3.6. Case Control Study 6: Ma & Jones, 2002 (20)

This study was carried out in Hobart, Southern Tasmania. It included 322 boys and girls aged 8 years. 32 children had fractures (cases), and there were 292 controls. This study was a nested case control study within a birth cohort originally recruited to investigate the long term implications of being at higher risk of Sudden Infant Death Syndrome (SIDS). 42% of the original cohort took part. All fractures occurred prior to the start of this nested case control study. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the lumbar spine, femur, and total body an unspecified length of time after fracture. Results showed no association between BMAD of total body adjusted for age, and fractures (mean in fractures 0.024g/cm^2 ± 0.001, mean in controls 0.024g/cm^2 ± 0.001, P=0.39). No evidence was seen for an association between BMAD of lumbar spine adjusted for age, and fractures (mean in fractures 0.10g/cm^2 ± 0.01, mean in controls 0.10g/cm^2 ± 0.01, P=0.99). No evidence was seen for an association between BMAD of neck of femur adjusted for age, and fractures (mean in fractures 0.34g/cm^2 ± 0.03, mean in controls 0.34g/cm^2 ± 0.05, P=0.67). The following methodological issues were identified:

- cases and controls were drawn from a previously recruited cohort set up to investigate sudden infant death syndrome
- cases and controls were comparable at baseline
- fractures were verified by X-ray or chart review where possible, but it is not stated how many cases were verified in this way
- bone density measured an unknown length of time after the fracture had occurred
- controlled for the potential confounding effects of body size by using BMAD
- children were assumed to be pre-pubertal because of their ages, but no formal assessment of pubertal stage was undertaken.

6.3.7. Case Control Study 7: Suuriniemi et al, 2003 (21)

This study was carried out in Jyvaskyla, Finland. It included 258 girls aged 10-12 years. 37 children had fractures (cases), and there were 221 controls. This was a nested case control study as part of a project set up to look at the effect of genetic polymorphisms in the COL1A2 gene on the risk of fractures in children. Children were from a cohort originally recruited for an intervention study to evaluate the effects of calcium, vitamin D and milk product supplementation on bone accrual. Exclusion criteria are listed in Table 27 on page 161. Bone density was measured by pQCT of the distal radius and tibia and estimated by size-adjusted DXA measurements of the lumbar spine, femur and total body. All fractures occurred more than 12 months prior to bone density measurements. Results showed an association between lumbar spine BMD adjusted for age, BMI and puberty and fractures not caused by severe trauma (mean in fractures 0.801g/cm^2 ± 0.08, mean in controls 0.835g/cm^2 ± 0.11, P=0.04). No evidence was seen for an association between lumbar spine BMC, total body BMD or BMC, neck of femur BMD or BMC, vBMD of distal radius or tibial shaft adjusted for age, BMI and puberty, and fractures. The following methodological issues were identified:

- cases and controls were drawn from a previously recruited cohort set up to evaluate the effects of calcium, vitamin D and milk product supplementation on bone accrual (the CALEX study), and this nested case control study was designed to investigate genetic associations of fractures in childhood
- cases and controls were comparable at baseline
- fractures were verified by X-ray or chart review where possible, but it is not stated how many cases were verified in this way
- bone density measured at least 12 months after the fracture had occurred
- adjusted for the potential confounding effects of body size by BMI in the analysis phase
- puberty was assessed by Tanner staging, and entry to the study was limited to participants who were pre-pubertal or in early puberty (Tanner stages I and II)
- multiple comparisons with increased likelihood that a 'significant' result will be seen by chance

6.3.8. Case Control Study 8: Ma & Jones, 2003 (22)

This study was carried out in Hobart, Southern Tasmania. It included 642 boys and girls aged 9-16 years. 321 children had fractures (cases), and there were 321 controls selected from the same school class as cases. Cases were recruited from a pre-existing fracture registry. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the lumbar spine, femur, and total body an average of 6 weeks after fracture. Results showed an association between total body BMD adjusted for age and weight and upper limb fractures (mean in fractures 0.89g/cm^2 ± 0.11, mean in controls 0.91g/cm^2 ± 0.11, P<0.01). There was an association between femoral neck BMD adjusted for age and weight and upper limb fractures (mean in fractures 0.76g/cm^2 ± 0.13, mean in controls 0.78g/cm^2 ± 0.14, P<0.05). There was an association between femoral neck BMAD adjusted for age and weight and upper limb fractures (mean in fractures 0.16g/cm^2 ± 0.01, mean in controls 0.17g/cm^2 ± 0.03, P<0.05). There was an association between lumbar spine BMD adjusted for age and weight and upper limb fractures (mean in fractures 0.75g/cm^2 ± 0.14, mean in controls 0.79g/cm^2 ± 0.16, P<0.01). There was an association between lumbar spine BMAD adjusted for age and weight and upper limb fractures (mean in fractures 0.11g/cm^2 ± 0.01, mean in controls 0.12g/cm^2 ± 0.02, P<0.01). No evidence was seen for an association between total body BMAD and upper limb fractures. The following methodological issues were identified:

- use of neighbourhood controls
- cases and controls were comparable at baseline
- fractures were verified by X-ray or chart review where possible, but it is not stated how many cases were verified in this way
- bone density measured 6 weeks after the fracture had occurred
- adjusted for the potential confounding effects of body size by weight in the analysis phase
- puberty was assessed by Tanner staging, and it was noted there was no difference in Tanner stage between cases and controls at baseline
- multiple comparisons with increased likelihood that a 'significant' result will be seen by chance

6.3.9. Case Control Study 9: Schalamon et al, 2004 (23)

This study was carried out in Graz, Austria. It included 204 boys and girls aged 8-12 years. 50 children had fractures (cases), and there were 154 controls selected from a local school. This case control study was specifically set up to test the hypothesis that bone mass in healthy children with fractures is lower than respective values in a control groups of healthy children without fractures. Exclusion criteria are listed in Table 27 on page 161. Bone status was measured by phalangeal, tibial and heel speed of sound (SOS) by QUS within 12 hours of fracture. Results showed the mean tibial SOS was 1914m/s ± 43 in children with fractures compared with 1928m/s ± 40m/s in children without fractures. The following methodological issues were identified:

- use of neighbourhood controls
- comparability of cases and controls at baseline not described
- no obvious verification of fractures
- bone density measured within 12 hours of fracture
- no adjustment for the potential confounding effects of body size in analysis or recruitment phase, but noted that there were no differences between the two groups in terms of weight
- no adjustment for the potential confounding effects of gender
- pubertal stage not assessed

6.3.10. Case Control Study 10: Goulding et al, 2004 (24)

This study was carried out in Dunedin, New Zealand. It included 50 boys and girls aged 0-13 years. 16 children had fractures (cases), and there were 34 controls. This was a nested case control study of bone density in cases and controls as part of a study into children with cow's milk avoidance. Exclusion criteria are listed in Table 27 on page 161. Bone density was estimated by size-adjusted DXA measurements of the lumbar spine, forearm, and total body an unspecified length of time after fracture. Results showed no evidence of an association between low Z score (volumetric bone density below –1SD) at the radius or lumbar spine adjusted for age and gender, and fractures (56% of fractures had a low Z score at lumbar spine compared with 32% of controls $P>0.05$; 38% of fractures had a low Z score at the distal radius compared with 47% of controls, $P>0.05$). The following methodological issues were identified:

- small numbers of children
- cases and controls were drawn from a previously recruited cohort set up to investigate the effects of cow's milk avoidance (recruited through advertisements)
- cases and controls were comparable at baseline
- fractures were verified by X-ray or chart review where possible, but it is not stated how many cases were verified in this way
- bone density measured an unknown length of time after the fracture had occurred
- controlled for the potential confounding effects of body size by using BMAD
- puberty was assessed by Tanner staging, and entry to study was limited to participants who were pre-pubertal or in early puberty (Tanner stages I and II)

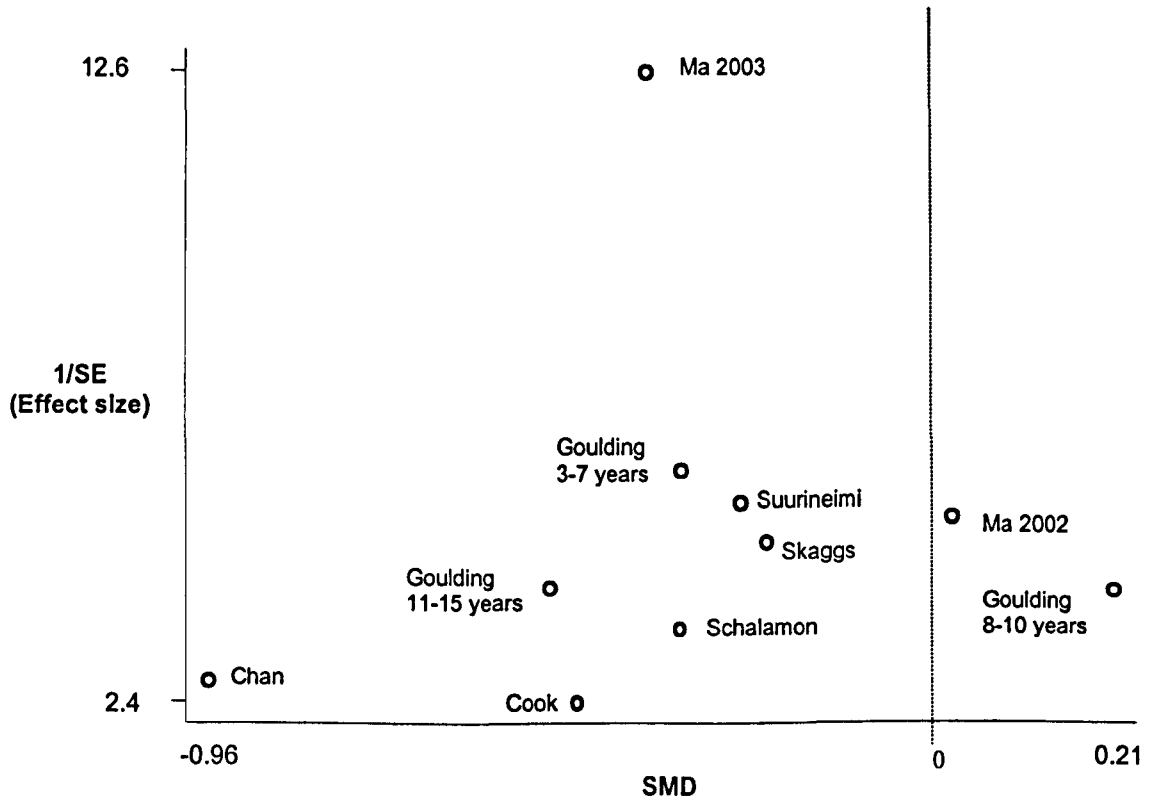
Table 31: Case control studies used in the systematic review of the association between bone mass and fracture risk in children

Author, year of publication	Geographical area covered, latitude	Age, gender	Cases (N of children with fractures)	Controls	Exclusions for cases and controls	Bone density measure	Time between fracture and bone density measurement
Landin 1982(15)	Malmo, Sweden, 59°N	4 to16 M & F	90	131 controls from same population as cases	Hand, finger, skull, tooth and rib fractures, metabolic bone disease, malnutrition, growth impairment	DXA of radius	40 days (± 25)
Chan 1984 (16)	Salt Lake City, Utah USA, 40°N	2 to12 M & F	17	17 unknown where drawn from	Existing chronic illness, malnutrition, underlying bone abnormalities	DXA non-dominant or non-fractured radius	16 months
Cook 1987 (17)	Louisiana, USA, 30°N	3 to14 M & F	17	17 unknown where drawn from	Metabolic bone disease, malnutrition, growth impairment, fractures of fingers, skull, teeth or ribs	DXA lumbar spine, left femoral neck	Within 4 weeks
Goulding 1998 (18)	Dunedin, New Zealand, 46°S	3 to15 F	100	100 controls (friends of cases with same age)	Non-Caucasian	DXA lumbar spine, left femur, total body, radius	Within 6 weeks
Skaggs 2001 (19)	Los Angeles, California, USA, 34°N	6 to15 F	50	50 matched community controls	Medium/high energy trauma fractures, chronic illness, ill > 2 weeks in last 6 months, previous hospitalisation, medications, vitamins, calcium supplements	CT radius	Within 1 month
Ma 2002 (20)	Southern Tasmania, 42°S	8 M & F	32	292 controls	Not at risk of Sudden Infant Death Syndrome	DXA of total body, lumbar spine and femur	unknown
Suuriniemi 2003 (21)	Jyvaskyla, Finland, 60°N	11 F	37	212 controls from same population as cases	History of serious medical conditions, medications known to affect bone, fracture < 1 year ago, serious trauma	DXA of total body, femur, lumbar spine pQCT of left distal radius Broadband attenuation left calcaneus by QUS	> 1 year
Ma 2003 (22)	Southern Tasmania, 42°S	9 to16 M & F	321	321 From same school class as a case	Diseases which may prevent them completing the study, moved out of area, not enrolled in school, previous upper limb fractures since aged 9	DXA total body, lumbar spine, right femoral neck	Average 6 weeks, max 3 months
Schalamon 2004 (23)	Graz, Austria, 48°N	9 to12 M & F	50	154 recruited from a school within same period	High impact trauma, illnesses except allergies	Speed of Sound of proximal phalanges of dominant hand measured by QUS	Within 12 hours
Goulding 2004 (24)	Dunedin, New Zealand, 46°S	0 to13 M & F	16	34 controls	No history of cows milk avoidance	DXA total body, lumbar spine and forearm	unknown

Abbreviations: CT computed tomography; DXA dual energy X-ray absorptiometry; F female; M male; N north; pQCT peripheral quantitative computed tomography; QUS quantitative ultrasound; S south

Eight of the studies presented results as means and standard deviations of bone density in cases and controls (16-23). The study by Landin and Nilsson (15) presented bone density of cases as percentage difference (cases minus controls). The study by Goulding et al 2004 (24) presented results as the percentage of children with volumetric bone density below one standard deviation of the study population. Using these eight studies (16-23) a Funnel Plot (Figure 18, below) was drawn and this showed no evidence of asymmetry.

Figure 18: Funnel plot of the 8 case control studies used in this systematic review and meta-analysis of the association between bone mass and fracture risk in children



Abbreviations: SE standard error; SMD standardised mean difference

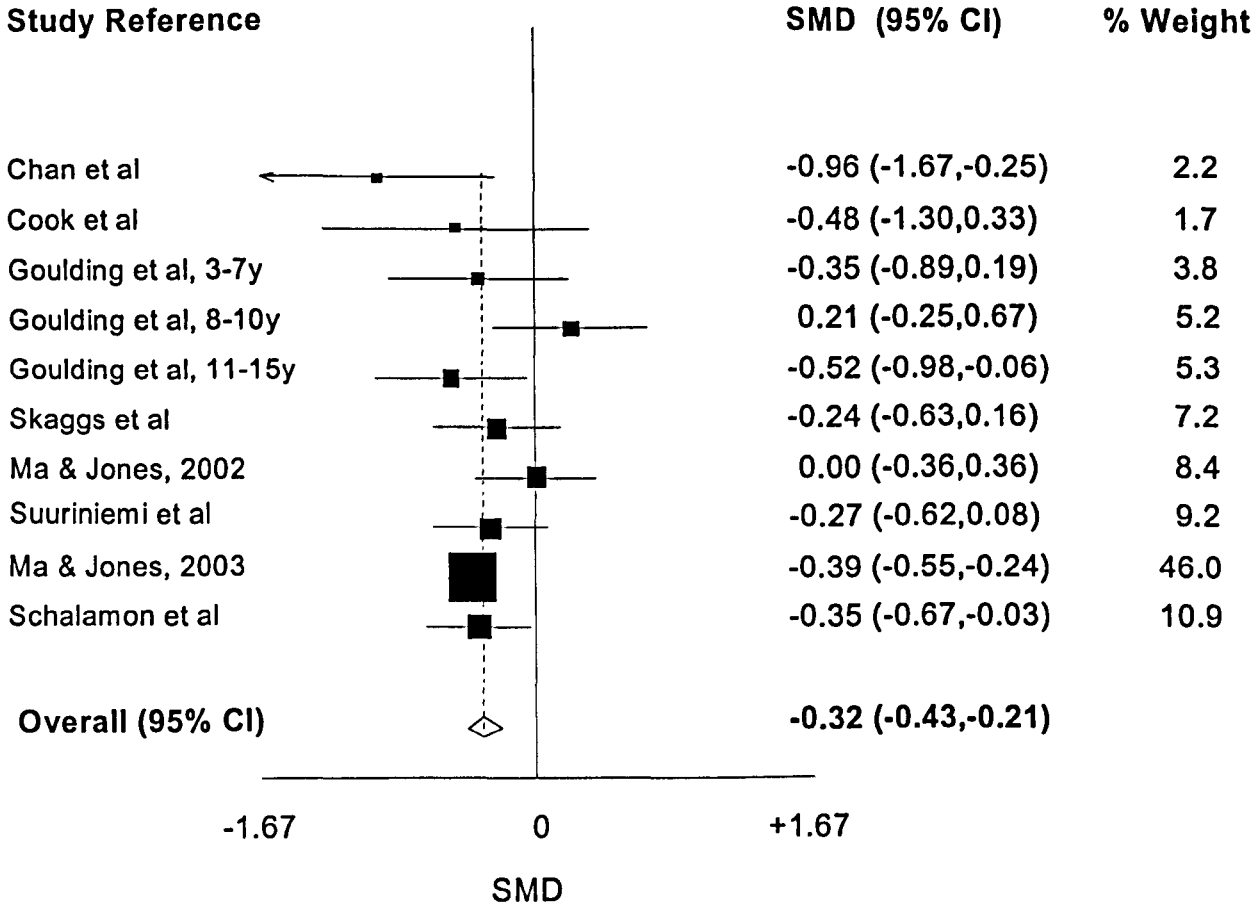
Formal testing of heterogeneity was carried out using the Chi-squared test, and this showed no evidence of heterogeneity (Chi-squared = 13.03 with 9 degrees of freedom, P=0.161). These eight studies were combined using a fixed-effects meta-analysis. As many of the studies presented multiple comparisons, estimates were chosen that

included a measure of body size, that used BMC and that used a peripheral measure of bone mass (forearm or femur) where possible. One study (18) presented data for three age groups of children, and results for these groups were included separately in the analysis.

The combined SMD in mean bone mass between children with fractures and controls was -0.32 (95% confidence interval -0.43 to -0.21, P<0.001). A forest plot is shown on page 164. The fixed-effects meta-analysis was repeated after excluding the largest study (22) and the results still showed an overall lower bone mass in children with fractures compared with controls (SMD -0.26, 95% confidence interval -0.40 to -0.11, P<0.001).

Further analysis was performed on the three studies that presented results for children with wrist and forearm fractures (18,19,22). This subgroup analysis showed a similar association to that observed in the main analysis with an SMD of -0.25 (95% confidence interval -0.40 to -0.10). When latitude of the study centres was assessed, the studies based further away from the equator were more likely to show an association between low bone mass and fractures in children.

Figure 19: Forest plot for fixed-effect meta-analysis of the association between bone mass and fractures in children based on eight case control studies



Abbreviations: CI confidence interval; SMD standardised mean difference

6.4. DISCUSSION

Ten case control studies, with a total of 730 fractures and 1328 control children, met the criteria for inclusion in this review. On combination of eight case-control studies the results show evidence of an association between low bone mass and fractures in children, with an SMD of -0.32 (95% confidence interval -0.43 to -0.21, P<0.001).

All of the studies were case control studies and are therefore prone to bias. In these studies, unclear verification of fractures may introduce bias as some 'cases' may not have fractured. This is possible in two studies (19,23) and would tend to move the observed association closer to the null. So the observed difference in mean bone mass between children with fractures and controls of -0.32 may be an underestimate. Lack of representativeness of the control selection may lead to a biased estimate of the effect of bone mass on fracture risk. However, most of the studies included in this review used accepted methods of control selection.

Confounding, both measured and unmeasured, is a problem in case control studies. In bone mass estimates made using DXA, adjusting for body size is important but difficult. If adjustment is not complete, this may lead to an inaccurate estimate of the effect of bone mass on fracture risk. There is no ideal technique, but all studies used at least one method to account for differences in body size, such as adjusting for height, weight or both, either during the recruitment or the analysis stage. Some studies noted there was no difference in either height or weight between the children with fractures and the control group (16-18,23). Two studies (20,24) used BMAD which is BMD corrected for area, and is less influenced by body size than either BMC or BMD. Other potential confounders that were considered by most studies were age and gender. The study by Schalamon (23) did not appear to adjust for gender, but direct communication with the lead author confirmed that there was no difference in gender between children with fractures and control group.

All of the studies measured bone mass in the cases after the bone fracture had occurred. This means that a reduction in bone mass due to the previous fractures cannot be excluded. However, repeat bone density measurements were taken on the children used in the study by Goulding 1998 (18) four years after the original fracture (451). This

showed a sustained lower bone mass in the children with fractures compared to those without. It is possible that behaviour is permanently modified by a fracture and results in a persistent low bone mass. However, the sustained low bone mass shown in the study by Goulding (451) is more likely to represent long-term bone mass and reduces the likelihood of reverse causality.

Multiple comparisons and subgroup analyses such as those carried out in the studies by Goulding 1998 (18) Suuriniemi (21) and Ma 2003 (22) increase the likelihood that a 'significant' result will be seen by chance. As the biggest weight in the fixed-effects meta-analysis was given to the study by Ma 2003 (22) this may mean these results are biased. However, repeating the fixed-effects meta-analysis without this study showed a similar difference in bone mass in children with fractures compared with controls. No asymmetry was shown by the Funnel Plot so publication bias is less likely, but the studies were small and six of the ten studies had positive results so we cannot exclude publication bias as an explanation.

In summary, the methodological quality of the studies included in this review were variable, with potential for bias and confounding. While the combined estimate should be interpreted with caution, these results suggest that bone mass may contribute to fracture risk in childhood. In adults, each standard deviation decrease in bone mineral density approximately doubles fracture risk (81). As most of the studies reported differences in mean values rather than differences in risk it is difficult to speculate how the results of this meta-analysis can be used to predict fracture risk in children. This systematic review and meta-analysis did not investigate the underlying causes for the association between bone mass and fractures in children, but geography may be important as the results suggested latitude influenced results, perhaps via cutaneous vitamin D synthesis. To investigate the association between bone mass and fractures in children further, large prospective cohort studies are required.

6.5. SUMMARY OF THE ASSOCIATION BETWEEN BONE MASS AND FRACTURES IN CHILDREN

- This systematic review and meta-analysis found evidence of an association between low bone mass and fractures in children, with an SMD of -0.32 (95% confidence interval -0.43 to -0.21, P<0.001)

- This results needs to be interpreted with caution as it is based on eight case control studies which are prone to bias and confounding

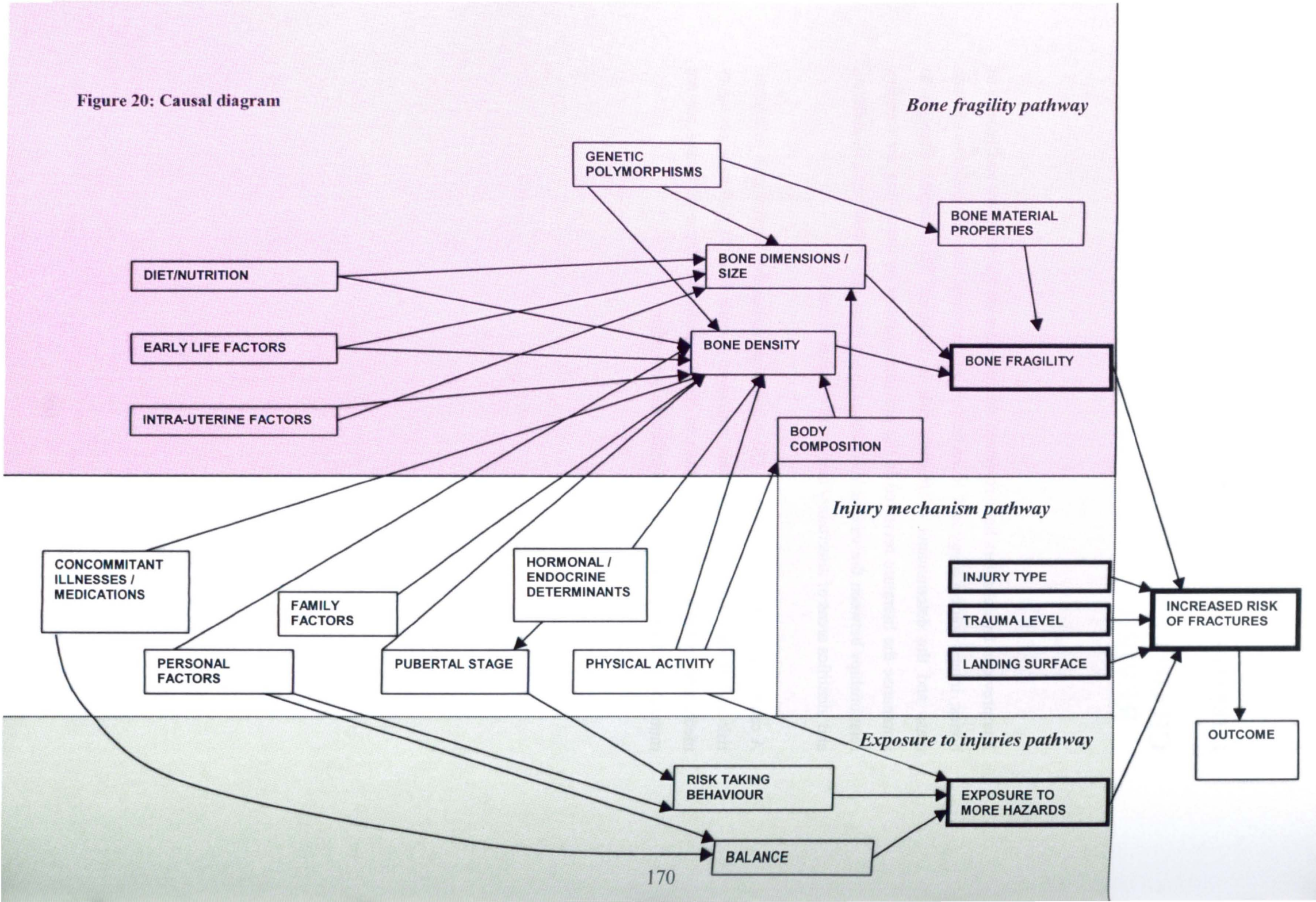
- Large prospective studies are needed to investigate further the association between bone mass and fractures in children

CHAPTER 7: SUMMARY OF LITERATURE REVIEW

The previous five chapters have reviewed the literature on the structure and growth of bones; injury epidemiology and identification of fractures; the determinants of bone mass and the determinants of fracture risk in children. This chapter attempts to summarise the literature reviewed in the previous chapters by describing the complex relationships between the various risk factors, bone mass and fracture risk in children, and identifies areas of uncertainty that require further research.

A causal diagram is shown on page 170, which summarises the determinants of fracture risk and divides them into three main pathways: a bone fragility pathway; an injury mechanism pathway; and an exposure to injuries pathway. These pathways are not mutually exclusive, and overlap and influence each other.

Figure 20: Causal diagram



7.1. SUMMARY OF THE ASSOCIATION BETWEEN BONE FRAGILITY AND FRACTURE RISK IN CHILDHOOD

The bone fragility pathway is shown in more detail in Figure 21 on page 172. In this figure the arrows represent the literature according to the epidemiological hierarchy of evidence (383). The heaviest arrows are for evidence from randomised controlled trials (RCTs), the next heaviest for cohort studies, then case control studies and finally cross-sectional studies. The broken arrows represent areas where no evidence is available or where evidence is contradictory.

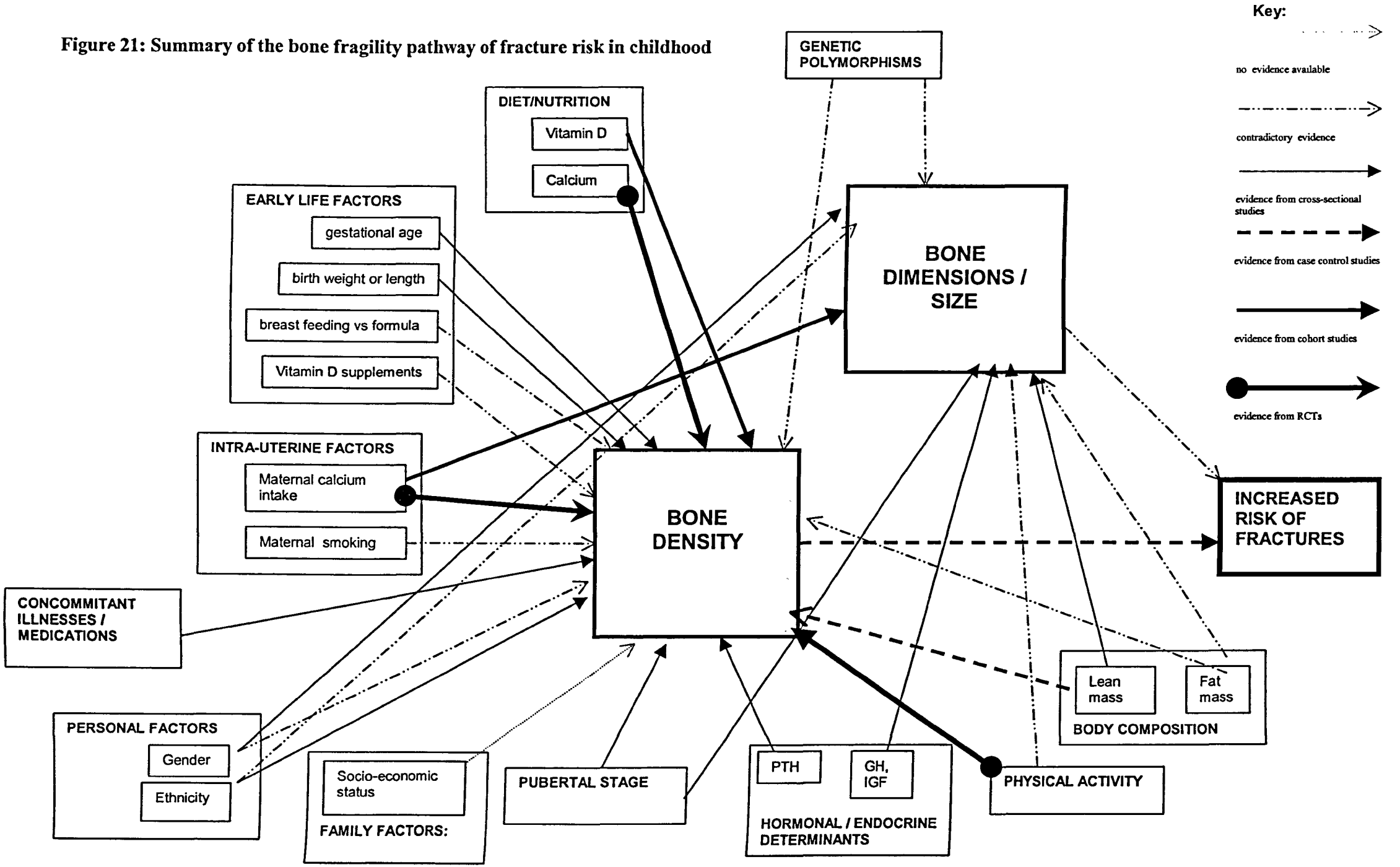
No evidence is available for the association between socio-economic status and bone mass in childhood. Contradictory reports are the only evidence available for the associations between genetic polymorphisms, maternal smoking during pregnancy, early life feeding regimes, early life vitamin D supplementation, gender or fat mass and bone density in childhood. Contradictory reports are the only evidence available for the associations between genetic polymorphisms, ethnicity, fat mass or physical activity and bone size in childhood. Also, contradictory evidence is available for the association between bone size and fracture risk in childhood.

Cross-sectional studies provide the best evidence for an association between gestational age, birth weight or length, ethnicity, pubertal stage and PTH and bone density in childhood. Cross-sectional studies also provide the best evidence for an association between gender, pubertal stage, GH or IGF and lean mass and bone size. Case control studies provide evidence for an association between lean mass and bone density in children, and for the association between bone mass and fracture risk in childhood.

Cohort studies show an association between vitamin D intake during childhood and bone density, and an association between maternal calcium intake during pregnancy and bone size in the offspring.

Randomised controlled trials provide evidence for an association between maternal calcium intake during pregnancy, childhood calcium intake and physical activity and bone density in childhood.

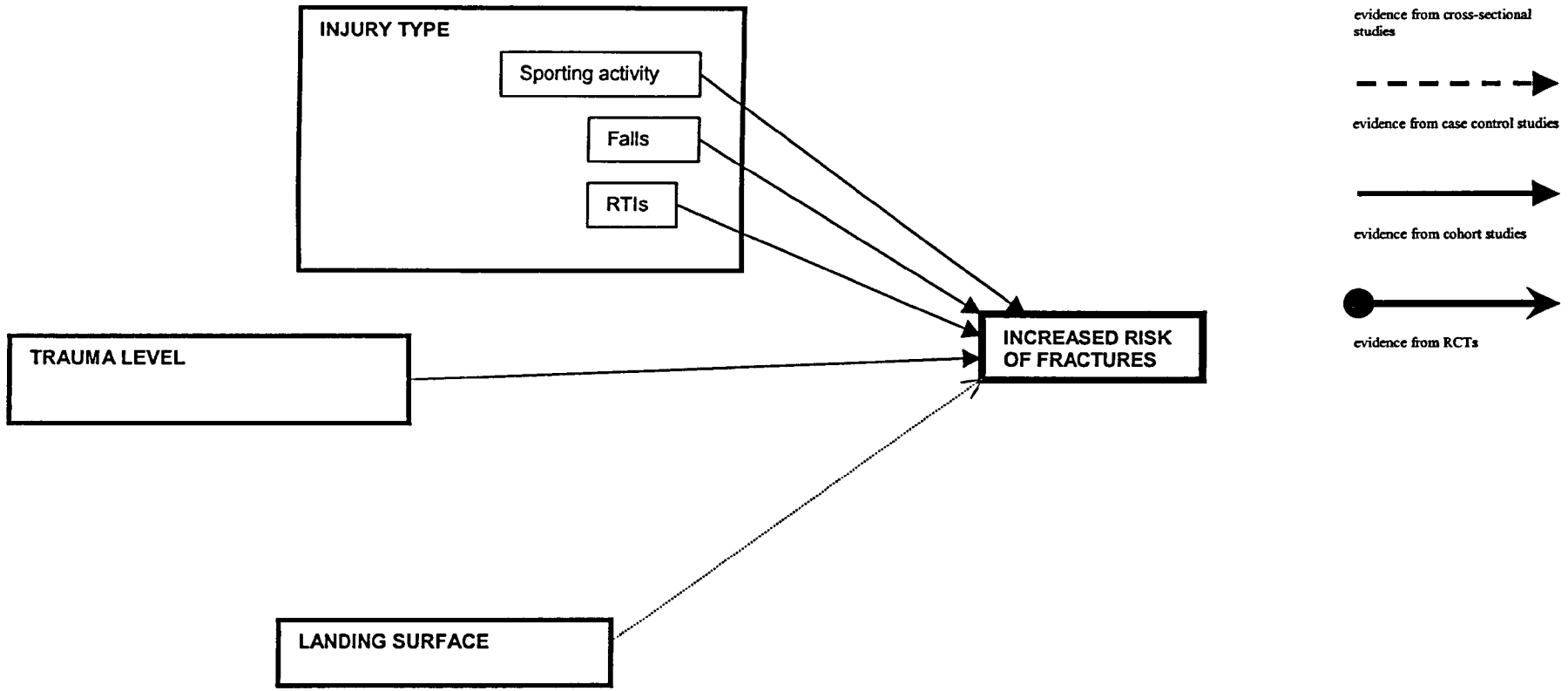
Figure 21: Summary of the bone fragility pathway of fracture risk in childhood



7.2. SUMMARY OF THE ASSOCIATION BETWEEN INJURY MECHANISM AND FRACTURE RISK IN CHILDREN

The injury mechanism pathway is shown in more detail in Figure 22 on page 174, with the same arrows as on the previous diagram. No evidence is available in the literature on the association between landing surface and fracture risk in children. Most of the reports on the association between injury type and fracture risk are cross-sectional studies and do not take account of time spent in the different activities. Cross-sectional studies provide evidence on the association between trauma level and fracture risk in children.

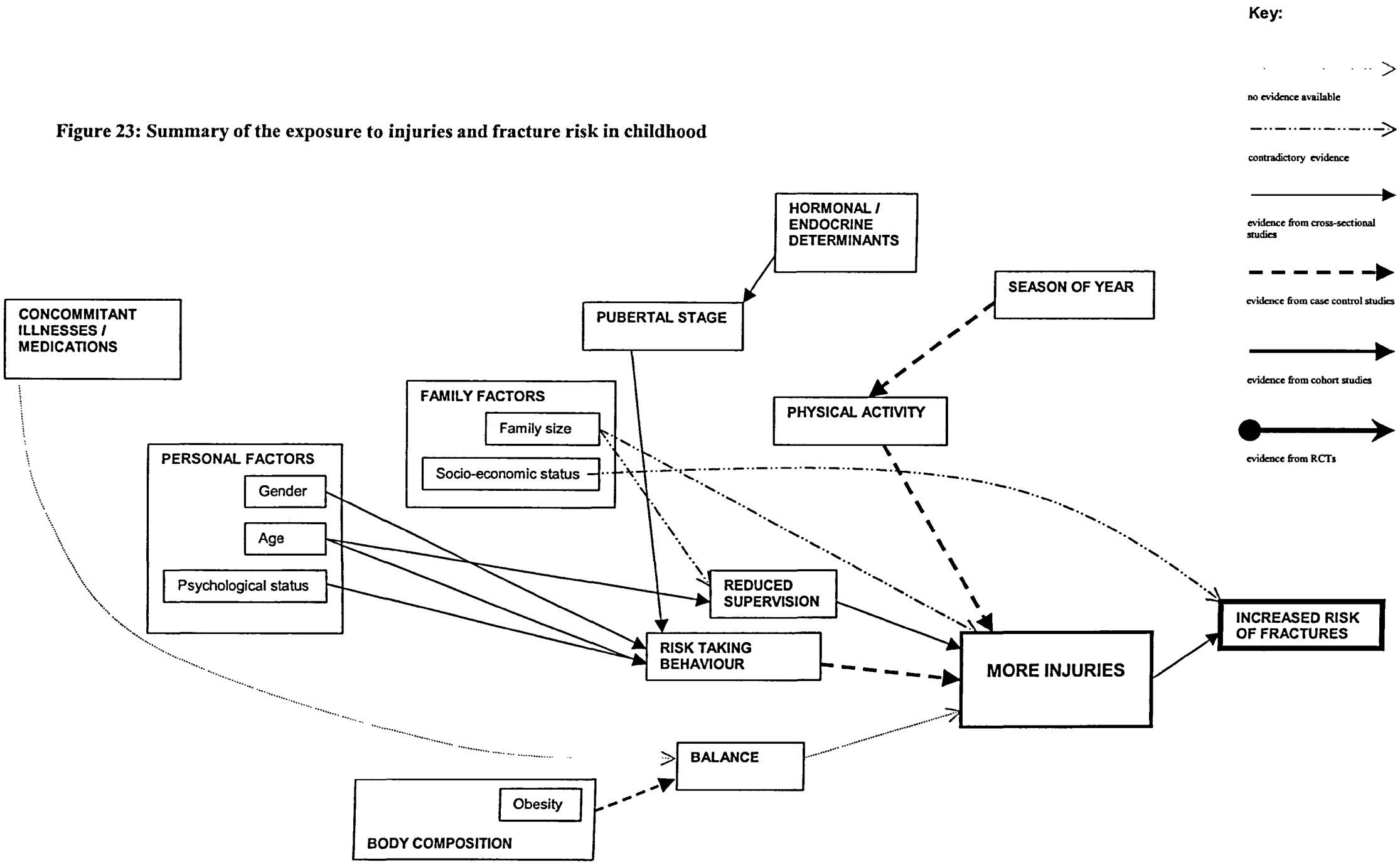
Figure 22: Summary of the injury mechanism pathway of fracture risk in childhood



7.3. SUMMARY OF THE ASSOCIATION BETWEEN EXPOSURE TO INJURIES AND FRACTURE RISK IN CHILDREN.

The exposure to injuries pathway is shown in more detail in Figure 23 on page 176. No evidence is available for the association between balance and fracture risk in children. Contradictory reports are the only evidence available for the associations between family factors such as family size and socio-economic status and fracture risk in childhood. Case control studies suggest that obesity is associated with reduced balance.

Figure 23: Summary of the exposure to injuries and fracture risk in childhood



7.4. SUMMARY OF THE DETERMINANTS OF FRACTURE RISK IN CHILDHOOD THAT NEED FURTHER RESEARCH

- Association between socio-economic status and bone mass
- Associations between genetic polymorphisms, early life feeding regimes, early life vitamin D supplementation, gender or fat mass and bone density
- Associations between genetic polymorphisms, ethnicity, physical activity or fat mass and bone size
- Association between bone density and fracture risk
- Association between bone size and fracture risk
- Association between landing surface and fracture risk
- Association between injury type and fracture risk
- Association between balance and fracture risk
- Associations between family factors and fracture risk
- Associations between injury rates and fracture risk in children
- Determinants of increased injury rates in children

CHAPTER 8: THE AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN (ALSPAC)

The methods section of this thesis is divided into three chapters. This chapter covers the general methods of the Avon Longitudinal Study of Parents and Children (ALSPAC), the next chapter (Chapter 9) describes the specific methods of the Fracture Study that I designed and ran, and the third methods chapter (Chapter 10) describes the general statistical methods used. The specific statistics are described in detail in the relevant results chapters.

8.1. OBJECTIVES OF THIS CHAPTER

- To present an overview of ALSPAC
- To place the Fracture Study within the context of ALSPAC
- To describe the methods of data collection in the main study relevant to this thesis

8.2. OVERVIEW OF ALSPAC

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort of mothers and children that recruited participants during pregnancy (www.alspac.bristol.ac.uk) (484). All pregnant women living in the Avon area with an expected date of delivery between 1st April 1991 and 31st December 1992 were eligible to join the study. Recruitment was through advertisements displayed in GP surgeries, libraries, local shops and pre-school playgroups; through the local radio and television stations; and through local midwives. In total, 14 541 pregnant women were enrolled and 14 062 children were born. The cohort has been followed closely from pregnancy with regular questionnaires, and from aged 7 years with regular physical and psychological assessments in a research clinic. The principle aim of ALSPAC is to investigate how genetic, biological, environmental, social and psychological factors interact over time to affect child health, behaviour and development. ALSPAC is

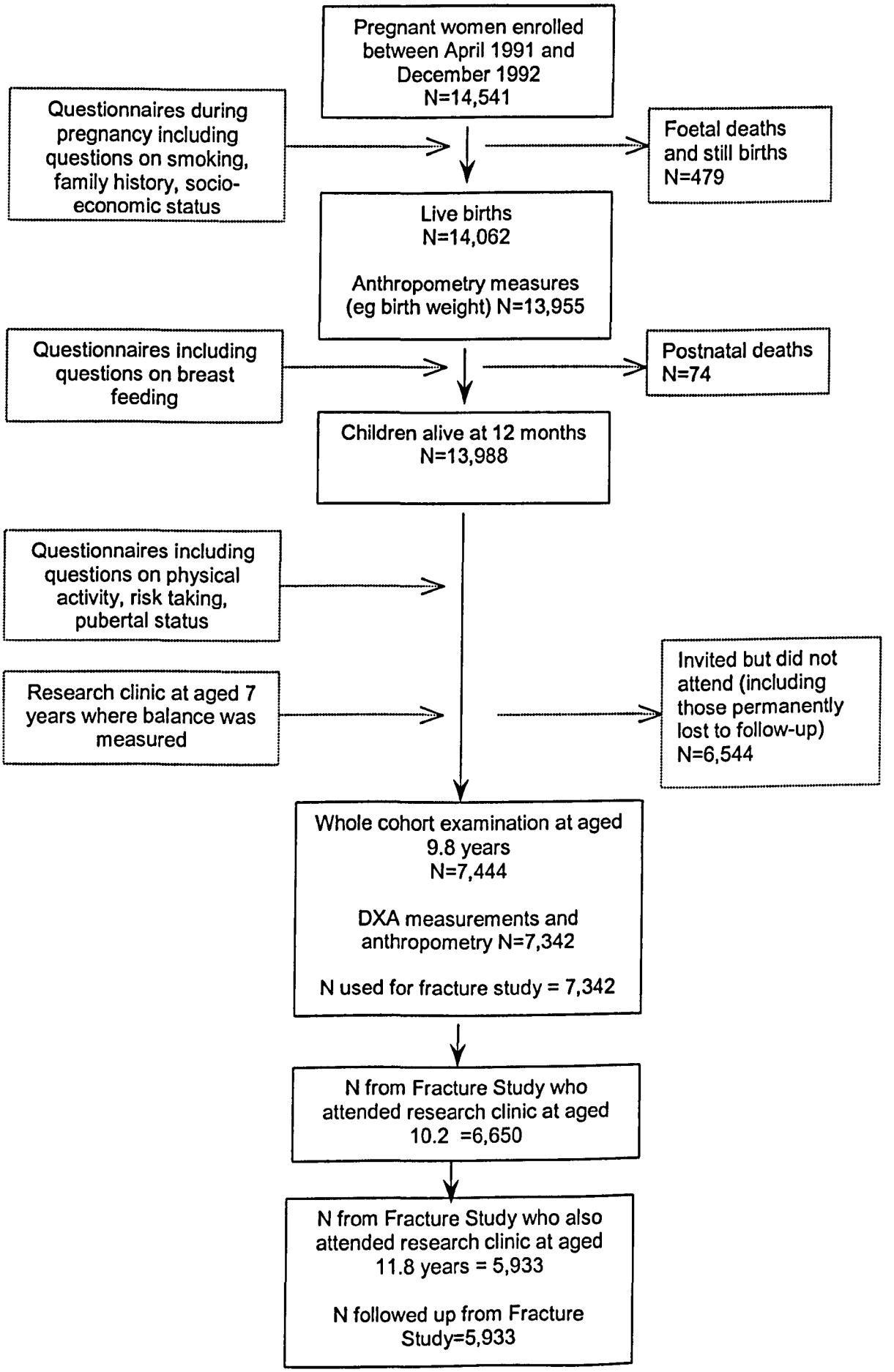
ongoing and aims to follow these children into adult life. At aged 9 years all children were invited to attend a research clinic for a DXA scan and other assessments, and 7444 attended.

ALSPAC was set up as part of a wider European Longitudinal Study of Parents and Children (ELSPAC), which was designed following a WHO-European meeting in 1985 on child health and development. ELSPAC also had study centres in Czech Republic, Russia, Slovakia, Ukraine and the Isle of Man. All ELSPAC study centres share the same core design and policy. All are observational population-based cohorts; the majority of data collection is through self-completed questionnaire by the mother and her partner linked to health records; the same core questions have been asked at all centres, allowing for culture-specific alternatives.

An overview of ALSPAC is shown in Figure 24 on the next page and highlights the main points of data collection relevant to this study. In pregnancy, data were collected by questionnaire from expectant-mothers at around 8, 12, 18 and 32 weeks gestation. Expectant-mothers were also asked to pass on questionnaires to their current partner. At birth, data were also collected from hospital birth records and from direct measurement of the study child by ALSPAC staff. The first post-natal questionnaires were sent out to study mothers at 4 weeks post-delivery. Since then regular questionnaires have been completed by both study mothers and partners enquiring both about the parents health and lifestyle and that of their child. As the children approach adolescence they are increasingly being asked to report data directly.

At aged 9.8 years the entire cohort of children were invited to attend a research clinic for examination. A component of this clinic included measuring body size and composition, by direct anthropological measurements (height and weight) and by DXA scan. All children were again invited to attend further research clinics at age 10.7 and 11.7 years, where they were asked if they had broken a bone since their DXA scan at aged 9.8 years. The Fracture Study is based on data from children who attended the three research clinics at aged 9.8, 10.7 and 11.7 years.

Figure 24: Overview of ALSPAC



8.3. ETHICAL APPROVAL

Ethical approval for ALSPAC has been obtained from the Southmead, Frenchay and Weston local Medical Research Ethics Committees, and the ALSPAC Law and Ethics Sub-committee. The ALSPAC Law and Ethics Sub-committee includes members of the ALSPAC study team, experts in law, ethics and child health from Bristol University, and representatives of the study mothers. The work of the ALSPAC Law and Ethics Sub-committee has been discussed in detail elsewhere (485,486).

8.4. ALSPAC STUDY FUNDING

The UK Medical Research Council, the Wellcome Trust and the University of Bristol provide core support for ALSPAC. ALSPAC has also received funding from the UK Department of Health, the UK Department of the Environment, the USA National Institutes of Health, and many charitable organisations and companies. The Fracture Study was specifically funded by a Wellcome Clinical Research Training Fellowship.

8.5. CONFIDENTIALITY

Confidentiality of the data collected in ALSPAC is maintained by the use of unique identification (ID) numbers, and by the separation of the data collection from the data analysis process. Each pregnant woman, her partner and foetus were given a unique ID number on entry to the study. This number links participants data longitudinally, and links parental and child data. Each questionnaire carries only this number and no other identifying information. Data collected at research clinics or abstracted from medical records are also kept separate from the participants' names.

8.6. REPRESENTATIVE NATURE OF ALSPAC

The general representativeness of the ALSPAC cohort compared to the population of the UK has been assessed previously using data from the 1991 census (www.alspac.bristol.ac.uk). Compared to all mothers with infants under one year of age in the UK, the ALSPAC mothers at 8 months post-delivery were more affluent (79.1%

versus 63.4% owned/mortgaged their own home, 90.8% versus 75.6% had a car in the household), more likely to be married (79.4% versus 71.8%), and more likely to be from a white ethnic background (97.8% versus 92.4%). This reflects the nature of the Avon population, and the characteristics of individuals who participate in epidemiological studies in general.

8.7. ALSPAC DATA USED IN THE FRACTURE STUDY AND METHODS OF DATA COLLECTION

8.7.1. General points regarding data collection and coding

In this section, the time of data collection reported is the average age of the study child at data collection, whether it be gestational age or actual age. For example, at the age 9 years research clinic the average age of the study children was 9.8 years, but some children were slightly older and some slightly younger. For questionnaires during pregnancy, the time of data collection indicates the first time the question was asked in a questionnaire. Women recruited later in pregnancy or at delivery were given modified versions of previous questionnaires to ensure all important data were collected. Therefore, for some questions during pregnancy, the time of data collection may have been a few months later than stated for some of the pregnant women recruited.

Data used in the Fracture Study, and the methods of data collection, are described chronologically, and are divided into child, maternal, paternal, family and socio-economic data. A summary of the data used in the Fracture Study and the age of the study child at collection is shown on page 196.

8.7.2. Child data

8.7.2.1. Demographic data

Gender

Data on gender were obtained from hospital birth records and birth notifications. This is a binary variable.

Ethnicity

Data on ethnicity were collected from the study mothers at 32 weeks gestation by self-completion questionnaire. They were asked to indicate their own and their partners ethnic background from the following list: white, black (Caribbean), black (African), black (other), Indian, Pakistani, Bangladeshi, Chinese or other ethnic group. If 'other' ethnic group was chosen, women were asked for a written description. Children were classified as of white ethnic origin if both parents were reported as white; black if both parents were black, or one parent was black and one was white; from the Indian sub-continent if both parents were from India, Bangladesh or Pakistan, or if one parent was from India, Bangladesh or Pakistan and one was white; Chinese if both parents were Chinese, or if one was Chinese and one was white; and 'other' if one or both parents were reported to be of mixed ethnicity or not from any other ethnic group.

For the purposes of the Fracture Study, data on ethnicity were recoded as a binary variable: white or non-white ethnicity.

8.7.2.2. Early life data

Gestational age

Gestational age was recorded as the gestation when the pregnancy ended. For all infants that were delivered after twenty weeks, if there was any suggestion that the delivery was preterm (i.e. before 37 weeks) then the clinical records were reviewed. Clinical records were reviewed not only for pregnancies that appeared preterm using the date of the mothers last menstrual period, but also if the clinically recorded gestation based on ultrasound, or the clinicians impression, was less than 37 weeks, or if the paediatric estimation of gestation was less than 37 weeks.

For the purposes of the Fracture Study, data on gestational age were used as either a continuous variable, or a binary variable: preterm birth (less than 37 weeks) or term birth (37 weeks gestation or older), based on the standard WHO's definition of premature birth from the International Classification of Diseases version 10 (ICD-10), codes P07.1 and P07.0 (455).

Birth weight

Data on birth weight were obtained in a variety of ways: from obstetric data; as recorded by the ALSPAC measurers; or from birth notifications. For the purposes of the Fracture Study, data on birth weight were used as either a continuous variable, or a binary variable: low birth weight (less than 2500 grams) or normal birth weight (2500 grams of heavier). This is the conventional WHO definition of low birth weight from the International Classification of Diseases version 10 (ICD-10), code P07.3 (455).

Breast feeding

Age of the study child in weeks, on stopping breast feeding, was asked by self-completion questionnaire at 6 months. A variable was derived by the ALSPAC study team which categorised the data into never breast fed, breast fed for less than one month, breast fed for one to less than 3 months, breast fed for 3 to less than 6 months, and breast fed for more than 6 months. This question did not ask about exclusive breast feeding, and the study children classified as breastfed may have received other feeds or milks such as formula milk.

8.7.2.3. Risk taking behaviour

Risk avoidance

Questions on risk avoidance were asked at 42 months (3.5 years). Specifically mothers were asked if their child never, hardly ever, sometimes, often or very often avoided taking risks. For the purposes of the fracture study this variable was reduced to three categories: never or hardly ever; sometimes; and often or very often.

8.7.2.4. Physical activity data

Balance and motor ability

To assess balance and motor activity, mothers were sent a questionnaire at 81 months (6.8 years) and the children were examined directly at aged 7.6 years in a research clinic.

In the questionnaire, mothers were asked about a range of skills and abilities that the child can do well, can do but not well, has not yet done, or is unable to do. For locomotor ability the skills asked about were

- the ability to walk,
- to stoop down and pick up something from the floor,
- to run,
- to jump forward with both feet together,
- to walk or run on tiptoe,
- to hop on one foot for three steps,
- to walk backwards for four steps,
- to stand on one foot for at least eight seconds,
- to walk upstairs putting both feet on one step or putting one foot on each step,
- to walk downstairs putting both feet on one step or putting one foot on each step,
- running upstairs one or two steps at a time,
- riding a bicycle with or without stabilisers,
- swimming with or without waterwings,
- doing a handstand against a wall or without support,
- skipping with a rope
- or standing on their head.

A composite score of locomotor ability was then derived by summing the number of items the child can do well (scored 2) and those the child can do but not well (scored 1). The total was divided by the total number of items minus the number the child had not had a chance to try. The composite score for locomotor ability was a continuous variables that was categorised into tertiles for the purposes of the Fracture Study.

At the research clinic at aged 7.6 years the children were asked to walk along a line drawn on the floor, walking heel to toe, and the number of correct steps out of 15 and the number of correct steps before an error were recorded. For the purposes of the Fracture Study these continuous variables were categorised into tertiles.

Time spent at various physical activity levels per week

Data on physical activity were collected by questionnaire at two time points: at 54 months (4.5 years) and 9 years. At 54 months questions were asked about average time in hours spent per week watching TV, spent in a vehicle, spent outdoors in summer and spent outdoors in winter. For the purposes of the Fracture Study the continuous variables time spent watching TV, in a vehicle and outdoors in winter per week were categorised into tertiles. Time spent outdoors in summer per week was reduced to a binary variable of none to 28 hours; or more than 28 hours per week.

At aged 9 years a question was asked about the average number of times the child had participated in vigorous physical activity over the last month and this was coded as none, less than once per week, 1 to 3 times per week, 4 to 6 times per week or daily. Vigorous physical activity was defined as activities such as running, dance, gymnastics, netball, swimming or aerobics. For the purposes of the Fracture Study this variable was reduced to three categories: less than 4 episodes per week; 4 to 6 episodes per week; or daily vigorous physical activity.

8.7.2.5. Dietary data

At 81 months a food frequency questionnaire (FFQ) was sent to all mothers. The answers to this were calibrated against the dietary record information obtained from a random 10% sub-sample (the Children in Focus, CiF, sub-sample) at aged 7 years in order to calculate the nutrient intakes. This was done by looking at the frequencies and total weights of foods eaten at aged 7 years by food group. These were used to generate a composite portion of food for each question on the FFQ, containing appropriate relative amounts of each of the most commonly eaten specific foods in the food group. For example, if 57% of the total weight of dark green leafy vegetables eaten at aged 7 years was broccoli, then the foods used to calculate the nutrient content of a portion of green vegetables in the FFQ was 57% broccoli by weight. The answers to the FFQ were

then used to create an approximate weekly nutrient intake for each child. This approximate weekly intake was calculated by multiplying the weekly frequency of consumption of a food by the nutrient content obtained from the 5th edition of McCance and Widdowson's 'The Composition of Foods' (487), and summing this over all foods consumed. Once a weekly nutrient intake had been calculated by this process it was divided by seven to give a daily nutrient intake.

For the purposes of the Fracture Study daily calcium, vitamin D and total energy intake were the variables chosen, as some evidence was available in the literature that these nutrients may be important for bone health (see Chapter 3, page 84). Daily calcium intake was measured in milligrams (mg) and was used as a continuous variable or as a categorical variable divided into quartiles. Daily vitamin D intake was measured in micrograms (μg) and was used as a continuous variable or as a categorical variable divided into quartiles. Daily energy intake was measured in megajoules (MJ) and was used as a continuous variable or as a categorical variable divided into quartiles.

8.7.2.6. Psychological status

At 91 months a questionnaire was sent to mothers that contained questions from the parent version of the Development and Well-Being Assessment (DAWBA) (488), which are aimed at making diagnoses from the Diagnostic and Statistical Manual of Mental Disorders, (DSM IV) (489). Based on the literature review (Chapter 5, Page 137) for the Fracture Study the diagnoses of attention-deficit hyperactivity disorder (ADHD) and oppositional/conduct disorder (OCD) were used. ADHD is defined by DSM IV (489) as symptoms of inattention that have lasted for at least six months to a degree that is maladaptive and inconsistent with developmental level, associated with symptoms of hyperactivity/impulsivity. There must also be clear evidence of clinically significant impairment in social, academic or occupational functioning. OCD is defined as (489) a repetitive and persistent pattern of behaviour in which the basic rights of others or major age-appropriate societal norms or rules are violated; or a pattern of negativistic, hostile and defiant behaviour lasting at least 6 months. Both ADHD and OCD were binary variables for presence or absence of these diagnoses.

8.7.2.7. Pubertal status

At aged 9 years a questionnaire was sent to mothers specifically to obtain details of Tanner staging of puberty (see Literature Review, Chapter 3, Table 7 on page 70 for a definition of the specific stages). Each questionnaire included a set of pictures of breast and pubic hair development for girls, and penis, scrotum and pubic hair development for boys. These were based on those developed by Tanner (169) and used in the USA. However, the boys pictures originally used by Tanner are shown with circumcised penises which are inappropriate for a British population, so these were changed. From ticks placed beside pictures each girl was assigned a Tanner stage (1 to 5) for both breast development and for pubic hair development. Boys were assigned a Tanner stage (1 to 5) for penis and scrotal development and for pubic hair development. The age of the child at completion of the Tanner staging questionnaire was also recorded.

For the purposes of the Fracture Study girls pubertal breast stage was divided into three categories: prepubertal (Tanner stage 1); early pubertal (Tanner stage 2); later pubertal (Tanner stages 3, 4 and 5). Girls pubertal pubic hair stage was also divided into the same categories. Boys pubertal penis and scrotum stage and pubic hair stage were divided into two categories prepubertal (Tanner stage 1) or pubertal (Tanner stages 2, 3, 4 and 5).

8.7.2.8. Data collected at the aged 9.8 years Research Clinic

DXA measurements

Total body DXA scans were performed using the Lunar Prodigy narrow fan beam whole body DXA scanner (GE Medical Systems, Lunar, Madison, USA), when the children were 9.8 years. Results for BMD (generated by dividing BMC by bone area, grams/cm^2), BMC (grams) and bone area (cm^2) are total body values less the head values (total body less head: TBLH). This is because the head is different to the rest of the skeleton in terms of bone development, and is not responsive to environmental stimuli such as physical activity (116). Using total body minus head derived data is likely to result in more sensitivity and predictive power (117). Data on fat mass (grams) and lean mass (grams) were total body values. Regional data can also be obtained from the Lunar software such as upper limbs, lower limbs or trunk.

Once a day the standard quality assurance tests using the calibration block were performed on the Lunar Prodigy. Once a week the radiation supervisor performed a spine phantom scan. In the event of failure of these tests, the software prevented further DXA scans being performed and the Lunar Support Team were contacted. All DXA operators were trained to the appropriate Ionizing Radiation (Medical Exposure) Regulations (IRMER) 2000 standard and have received the relevant certificate.

The child's height, weight, date of birth, gender and ethnicity were entered into the DXA computer by clinic staff. Where practical, metal objects such as bracelets and watches were removed before scanning. The child was positioned carefully on the bed, ensuring separation between the legs and between the arms and the trunk. The child was asked to lay their hands flat on the bed and to lie as still as possible. The scan itself was automatic with the scan parameters being determined by the software. When technical problems with the scanner occurred which prevented a complete scan, a second attempt was made immediately. If the problem persisted or the scanner was inoperative, the child was invited back to the clinic specifically to have a DXA scan. Of the 7444 scans performed at aged 9.8 years, 102 results could not be used because of scanner related error or artifacts.

Although the analysis software made an attempt to define the various skeletal regions automatically, these were re-analysed, as it is common practice to do, to achieve consistent boundaries between the regions. 122 scans were repeated on the same day, and this allowed an estimate of the variation between repeat DXA scans to be calculated. The coefficient of variation for total body bone mineral content minus head was 0.8%.

For the purposes of the Fracture Study TBLH BMC, BMD and BA were used as continuous variables. TB fat and lean mass were used both as continuous variables and categorical variables divided into quartiles.

Anthropometric measurements

Height

Height was measured at the time of the DXA scan. It was measured to the last complete mm using the Harpenden Stadiometer (Holtain Ltd, Pembrokeshire, UK). Children were positioned with their feet flat and heels together, standing straight so their heels, calves, buttocks and shoulders came into contact with the vertical backboard of the stadiometer. The headboard was lowered down the blackboard until it touched the child's head and a 1 Kg weight was placed on the headboard to ensure head contact and to minimize the effect of hair thickness. The child was asked to relax their shoulders and stretch up but keeping their heels in contact with the ground. For the purposes of the Fracture Study height was used both as a continuous variable and a categorical variable divided into quartiles.

Weight

Weight was measured at the time of the DXA scan. It was measured using the Tanita Body Fat Analyser model TBF 305 (Tanita UK Ltd, Yewsley, UK). The child was encouraged to pass urine and to undress to their underclothes. The child stepped onto the measuring platform and weight was measured to the nearest 50g. For the purposes of the Fracture Study weight was used both as a continuous variable and a categorical variable divided into quartiles.

Body Mass Index (BMI)

BMI was calculated from height and weight using the formula

$$\frac{\text{Weight in kilograms}}{(\text{Height in metres}) \times (\text{Height in metres})}$$

8.7.3. Maternal data

8.7.3.1. Size of pregnancy

Data on whether each child was part of a multiple or singleton birth was recorded during pregnancy. No distinction was made between twins, triplets or quadruplets. Nor was any distinction made between those multiple pregnancies for whom there was one survivor as opposed to those where there were no survivors or many survivors. A binary variable was used: singleton pregnancy or multiple pregnancy.

8.7.3.2. Maternal smoking during pregnancy

Data on maternal smoking in pregnancy were collected at 18 weeks gestation. Women were asked if they had smoked in the two weeks prior to completion of the questionnaire, and to record how many cigarettes they had smoked per day. For the purposes of the Fracture Study, data on maternal smoking during the second trimester of pregnancy were used as a binary variable: yes or no. Other questions on maternal smoking are available but have not been used in the Fracture Study.

8.7.3.3. Maternal body size

Data on maternal height and pre-pregnancy weight were collected from expectant mothers by questionnaire at 12 weeks gestation. Weight was reported in either stones and pounds or kilograms, and all data were converted to kilograms by multiplying the values in pounds by 2.2046. Height was reported in either feet and inches or centimetres, and all data were converted to centimetres by multiplying the value in inches by 2.54. For the purposes of the Fracture Study maternal weight and height were used as continuous variables or categorised into tertiles. BMI was calculated from height and weight using the formula

$$\frac{\text{Weight in kilograms}}{(\text{Height in metres}) \times (\text{Height in metres})}$$

8.7.3.4. Maternal history of fractures

Data on maternal history of a previous broken arm/hand or broken leg/foot was collected during pregnancy. Mothers were asked whether they were hospitalised, saw a doctor as an outpatient or were treated at home only for the fracture. For purposes of the Fracture Study, data were coded as two dichotomous variables: yes fractured in the past or no history of fracture, separately for the upper and lower limb.

8.7.3.5. Maternal age

Data on maternal age at delivery of the study child were collected from questionnaires completed just after birth. Age was reported in years. For the purposes of the Fracture Study, maternal age was used as either a continuous variable or categorised into tertiles.

8.7.4. Paternal data

8.7.4.1. Paternal body size

Mothers were asked during pregnancy about their partners weight and height. Data on paternal height and weight was collected in the same manner as described for maternal body size on page 192.

8.7.4.2. Paternal history of fractures

Mothers were asked during pregnancy about whether their partner had ever broken an arm/hand or leg/foot. Data on paternal history of fractures was collected in the same manner as described for maternal history of fractures above.

8.7.5. Family data

8.7.5.1. Family size

At 47 months (3.9 years) mothers were sent a questionnaire which included questions on how many people lived in their household, including themselves, how many were over 18 years, how many were young adults aged 16 to 18 years and how many were

children aged less than 16. For the purposes of the Fracture Study, the data on family size used was the total number of persons in the household. This was reduced to a variable with three categories: 1 to 3 people; 4 people; or more than 4 people in the household.

8.7.6. Socio-economic data

8.7.6.1. Housing tenure

At enrolment to the study mothers were asked using a self-completion questionnaire whether their home was

- being bought/mortgaged,
- owned with no mortgage to pay,
- rented from the council,
- rented from a private landlord furnished or unfurnished,
- rented from a housing association
- or other.

For purposes of the Fracture Study this was reduced to three categories: mortgaged/owned, private rental or council/housing association rental.

8.7.6.2. Parental education

At 32 weeks gestation mothers were asked using a questionnaire what educational qualifications they and their partner had. They were asked to tick all that applied from a list of

- Certificate of Secondary Education (CSE) or General Certificate of Secondary Education (GCSE) at grades D, E, F or G;
- O-level or GCSE at grades A, B or C;
- A levels;
- qualifications in shorthand/typing/other skills such as hairdressing;
- apprenticeship;
- state enrolled nurse;
- state registered nurse;

- City & Guilds intermediate technical;
- City & Guilds final technical;
- City & Guilds full technical;
- teaching qualification;
- university degree;
- no qualifications;
- and other qualifications.

A variable was then derived for mothers and partners highest achieved educational qualification and coded as five categories: CSE/none, vocational, O-level, A-level and degree.

8.7.6.3. Parental social class

At 32 weeks gestation mothers were asked using a questionnaire about their current occupation and that of their partner. They were asked to describe the actual job, occupation, trade or profession, using precise terms such as radio mechanic, woodworking machinist, tool room foreman. If the occupation is known by a special name, they were asked to use that name. If they or their partner were in H. M. Forces, they were asked to give the rank in addition to the actual job. Descriptions were also asked for the type of industry or service given: i.e. what is made, materials used, or services given. From the answers above, the mothers and partners occupation was coded using the 1991 OPCS classification, and the mothers and partners social class categorisation was derived as I, II, III non-manual, III manual, IV and V. HM Forces were given a separate code. For the purposes of the Fracture Study, maternal and paternal social class were used separately, with the HM Forces code dropped and classified as missing. People unemployed at the time of the questionnaire were asked to complete the question based on their last main occupation.

8.7.7. Summary of data used and timing of data collection

A summary of the data used in this project and the time of data collection is shown in Table 32 on the next page.

Table 32: Summary of the data used in this project and the time of data collection

	Pregnancy[a]	Birth	6 months[a]	42 months[a] (3.5 years)	47 months[a] (3.9 years)	54 months[a] (4.5 years)	81 months[a] (6.8 years)	91 months[a] (7.6 years)	7.6 years[b]	9 years[a]	9.8 years[b]
Child data											
Gender		✓									
Ethnicity		✓									
Gestational age		✓									
Birth weight		✓									
Breast feeding			✓								
Risk avoidance				✓							
Physical activity						✓				✓	
Balance							✓		✓		
Dietary data							✓				
Psychological status								✓			
Pubertal status										✓	
Age at DXA scan											✓
TBLH BMD											✓
TBLH BMC											✓
TBLH bone area											✓
TB fat mass											✓
TB lean mass											✓
Height											✓
Weight											✓
BMI											✓

[a] data from questionnaires sent to mother

[b] data collected directly from the children at research clinics

Abbreviations: BMC bone mineral content; BMD bone mineral density; BMI body mass index; DXA dual energy X-ray absorptiometry; TB total body; TBLH total body less head

Table 32, continued

	Pregnancy[a]	Birth	6 months[a]	42 months[a] (3.5 years)	47 months[a] (3.9 years)	54 months[a] (4.5 years)	81 months[a] (6.8 years)	91 months[a] (7.6 years)	7.6 years[b]	9 years[a]	9.8 years[b]
Maternal data											
Size of pregnancy	✓										
Smoking in preg	✓										
Weight	✓										
Height	✓										
History of fractures	✓										
Age at delivery		✓									
Paternal data											
Weight	✓										
Height	✓										
History of fractures	✓										
Family data											
Family size					✓						
Socio-economic data											
Housing tenure	✓										
Parental education	✓										
Parental social class	✓										

[a] data from questionnaires sent to mother

[b] data collected directly from the children at research clinics

CHAPTER 9: THE FRACTURE STUDY

The previous methods chapter discussed the general methods of ALSPAC. This chapter outlines my personal contribution to this study and describes the study design and methods of the Fracture Study.

9.1. MY ROLE IN THE FRACTURE STUDY

I formally joined ALSPAC in September 2003, but had been peripherally involved since 2001. As stated in the authors declaration I helped design the Fracture Study, managed the Fracture Study, reviewed the literature, designed, piloted and sent out the information leaflet and consent forms, obtained copies of X-ray reports to verify fractures, cleaned the data, developed a novel method for measuring long-bone dimensions from whole body DXA scans, and performed all statistical analyses with support from Mr Colin Steer, Dr Andy Ness and Dr Jon Tobias. The Fracture Study was developed from a discussion between myself, Dr Jon Tobias and Dr Andy Ness on the measurement of bone mass in ALSPAC. ALSPAC clinic staff collected data on reported fractures in research clinics. Members of the Family Liaison Team, particularly Mrs Glynda Tanner, collated the names and addresses of children and their parents who reported any fracture. Mr Andy Boyd developed a database to allow me to keep track of consent form deployment, reminder letters and returns.

9.2. FRACTURE STUDY OVERVIEW

The Fracture Study consisted of three parts: the main analytical study; the development of a novel method of obtaining long-bone geometrical parameters from total body DXA scans; and verification of fractures by obtaining X-ray reports (Figure 25, page 201). My work on the study started in September 2003 and continued until March 2006, although data were collected from February 2002.

At aged 9.8 years the children were invited to attend a research clinic where they had a DXA scan. This research clinic ran from January 2001 to January 2003. On subsequent

attendances at a research clinic approximately 12 months after their DXA scan, which ran from February 2002 to October 2003, and a research clinic approximately 24 months after their DXA scan, which ran from January 2003 to January 2005, data were collected on whether each child reported a fractured bone since their DXA scan. A database was produced by mechanically scanning completed research clinic forms. This database indicated whether each child reported a fracture since their DXA scan or not. This database was linked with data previously collected as part of the main ALSPAC study (Methods, Chapter 8, Table 32, page 196) to allow statistical analysis.

A novel method was developed to obtain measures of humeral shape from total body DXA scans. Two methods were piloted on ten scans to obtain a measure of repeatability and variability. Validity was then assessed on a random 5% sub-sample. The method was then applied to the DXA scans of all children who reported a fracture, and a random sub-sample of children who did not report fractures. The new data were linked with the fracture data and with data previously collected as part of the main ALSPAC study (Methods, Chapter 8, Table 32, page 196) to allow statistical analysis.

A consent form was sent to children who fractured to obtain copies of the X-ray report to allow verification of the fracture. After ethical approval had been obtained in November 2004 (see page 202), the method of obtaining copies of the X-ray reports was piloted in a local NHS fracture clinic. In May 2005 the consent forms were sent out to the children who reported fractures since their DXA scans. Forms were sent out in batches of 50 to ensure good tracking, and to allow the Family Liaison team to make sure specific children and their parents were not being overloaded with other contacts from ALSPAC in a short space of time. Reminder letters with further consent forms were sent out if no reply had been received within four weeks. The last batch of consent forms was sent out in January 2006. X-ray reports were obtained if the original X-ray had been taken at one of three local NHS trusts, and data were extracted on whether or not a fracture had been reported to allow verification.

A questionnaire asking about details of the fracture was enclosed with the consent form (see Appendix B, page 408), but due to time constraints has not been analysed as part of this PhD.

Figure 25: The Fracture Study time line

Date	Analytical Study	Novel Method Development	Fracture Verification
Feb 2002	Children asked whether they have fractured since their DXA scan 12 months previously		
Jan 2003	Children asked at whether they have fractured since their DXA scan 24 months previously		
Mar			
May			
July			
Sep	Start of general literature review		
Nov			
Jan 2004	Start of analysis of confounding structure of bone mass in children		
Mar		Development of method for measuring humerus geometry - piloted on 10 scans	Start of design of method to obtain copies of X-ray reports for verification of fractures
May			
July	Start of analysis of fracture determinants		
Sep		Method finalised, validation of results, repeatability measured	Ethical approval obtained for obtaining copies of X-ray reports for verification of fractures
Nov			
Jan 2005		Humerus geometry measured on a sample of 1557 children	
Mar			Piloted method of obtaining X-ray reports
May			
July			Started sending out consent forms to obtain copies of X-ray reports
Sep			
Nov			Finished sending out consent forms
Jan 2006			
Mar	Completion of analysis of fracture determinants including humerus geometry		Verification of fractures

9.3. ETHICAL APPROVAL

Ethical approval for the main Fracture Study was obtained from the ALSPAC Law and Ethics Committee. Ethical approval for piloting in an NHS clinic and for obtaining copies of X-ray reports from NHS institutions for research purposes was granted by the Central and South Bristol Local Research Ethics Committee (CSB LREC), as part of the Central Office for Research Ethics Committees (COREC). CSB LREC also granted ethical approval for the main Fracture Study. See Appendix C, page 416.

9.4. ANALYTICAL STUDY

The main analytical component of the fracture study comprises three sections: the descriptive epidemiology of the ALSPAC variables used; the analysis of the confounding structure of bone mass in children at aged 9.8 years; and an analysis of the association between estimated volumetric bone density or bone size measured at aged 9.8 years and fracture risk over the following two years. The analytical study and statistical methods used are discussed in further detail in the next chapter on page 219.

9.5. REGIONAL ANALYSES OF THE HUMERUS FROM TOTAL BODY DXA SCANS

9.5.1. Introduction

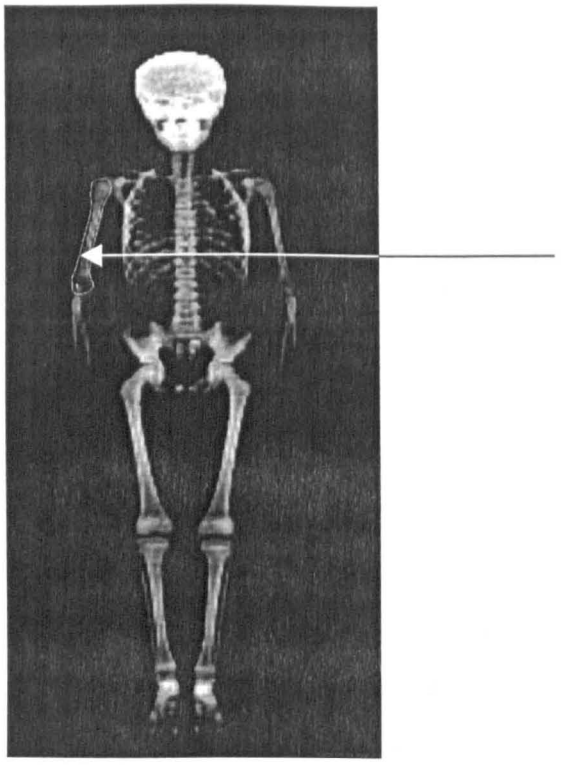
One explanation for the increased risk of fractures that is seen during puberty (see Literature Review, Chapter 5, page 133), is that growth of long-bones occurs lengthways before widthways i.e. longer forearms may have relatively narrower radial cross-sections and be at an increased risk of fracture. As measurements of bone mass produced by the DXA scanner are not true three-dimensional density measurements, but a two-dimensional estimate, it is usual to correct these estimates for height and weight to reduce size-related artefacts (see Literature Review, Chapter 2, page 48). However, when looking at associations between bone mass and fracture risk, it may be that also including a measure of long-bone geometry would provide a better model for predicting which children are at an increased risk of fracture.

9.5.2. Method of producing humeral geometrical measurements

Starting from a Total Body DXA image, the 'Custom' option was selected from the 'Analyze' pull-down menu. The upper limb was then enlarged to the maximum size possible. From the tool bar 'ROIs' (Region of Interests) was selected. An icon was selected from the left hand tool bar that allowed creation of a non-standard shape. This shape was then fitted around the humerus as closely as possible to both the shaft, head of humerus and elbow (Figure 26 on the next page).

The button 'Results' was clicked to get BMD, BMC and area. Width and length of this shape was not used, as this represented the width and length of the overall vertical area covered by the shape, and was dependent on the angle of the arm. A further ROI (ruler function) was placed stretching from the top to the bottom of the humerus, and the length of this line was noted. Average width was therefore calculated as area divided by length. Percentage fat and lean were not used as this was dependent on how closely the ROI was drawn around the shaft of the humerus.

Figure 26: Total body DXA scan showing Region of Interest drawn around the right humerus



9.5.3. Precision study

9.5.3.1. Methods of precision study

10 scans were randomly selected. The measurements were repeated 5 times on each scan, 6 hours apart. The mean, standard deviation (SD) and coefficient of variance (CV) were calculated. The CV is a standardisation of the SD that allows comparisons of variability estimates, regardless of the magnitude (490) of BMC or bone width etc. It expresses the SD as a percentage of the mean. See Table 33 below for the mean inter-scan coefficient of variation for each measurement.

Table 33: Summary of the mean inter-scan coefficient of variation (CV) for all measurements

Variable	Mean	SE	95% CI
Width (cm)	2.00	0.25	1.51, 2.50
Length (cm)	0.94	0.15	0.65, 1.23
Area (cm^2)	2.39	0.38	1.65, 3.14
BMD (g/cm^2)	0.68	0.13	0.43, 0.93
BMC (g)	2.04	0.19	1.67, 2.41

9.5.3.2. Conclusion of precision study

The mean CV for width is 2.0% which is small. The 95% confidence intervals are narrow. The measures of width and length from total body DXA scans using the developed method are precise.

9.5.4. Validation study

Humerus area, length, BMD and BMC were measured on the whole body DXA scans of 389 out of 7342 scans (5.3%). These 398 were chosen using a random number generator on a hand-held calculator to give the start position on the list of 7342 scans. The random number generator was then used to select the next scan to be used. Humerus width was then calculated as area divided by length. The humeral geometric variables and humeral BMD and BMC were plotted, and their distribution examined (see below).

This dataset was then linked to existing data on whole body DXA variables, anthropometric variables and puberty. To assess the validity of the humeral measures obtained the following hypotheses were investigated:

- there is an association between the geometric measures and gender, specifically boys have larger bones than girls
- humeral area, length, width, BMD and BMC increase with age
- there is an association between the humeral measures and measures of body size such as height and weight
- there is an association between humeral BMD and BMC and total body BMD and BMC
- humeral area, length, width, BMD and BMC increase with increasing pubertal stage

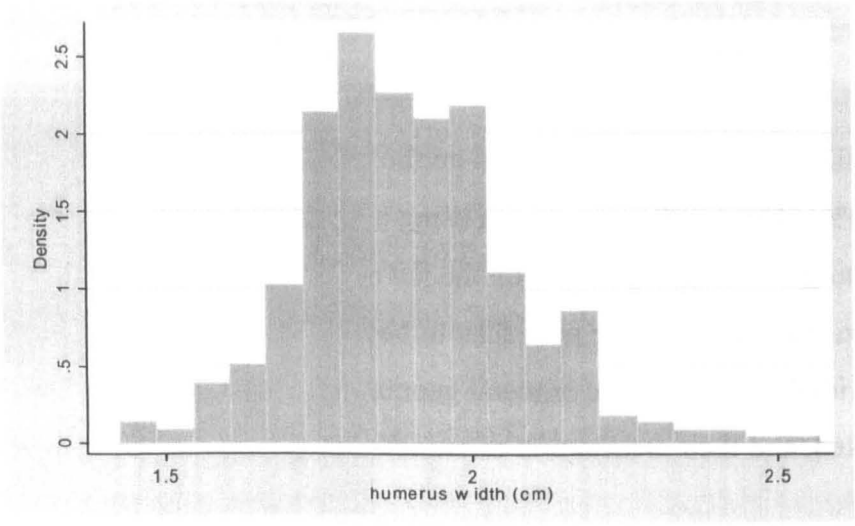
9.5.4.1. Distribution of the humeral variables

For the humeral variables width, length, area, BMD and BMC, their distribution was assessed using STATA 8.0 (see Methods, Chapter 10, page 219 for further details on this statistics package). A frequency histogram was drawn for each variable with frequency on the Y axis and values for each variable on the X axis. A summary of the

mean and SD for each variable overall and specifically for boys and girls is shown in Table 34, page 209.

Humerus width

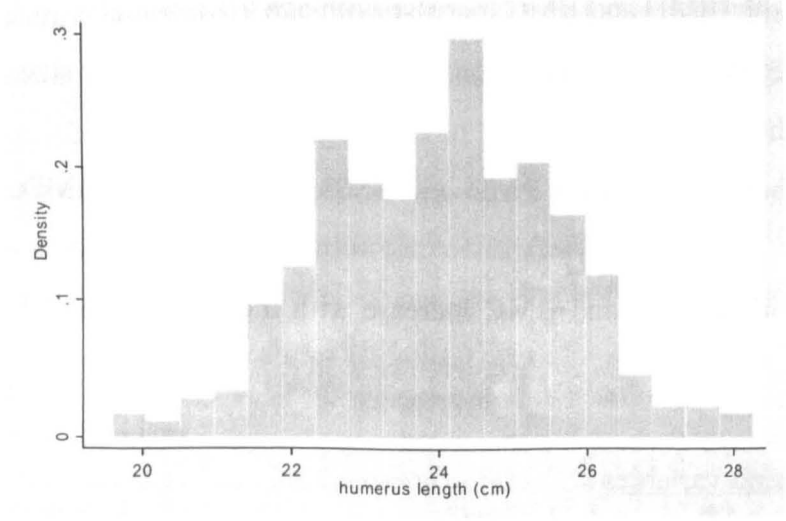
Humerus width is approximately normally distributed:



mean 1.90 cm
SD 0.17

Humerus length

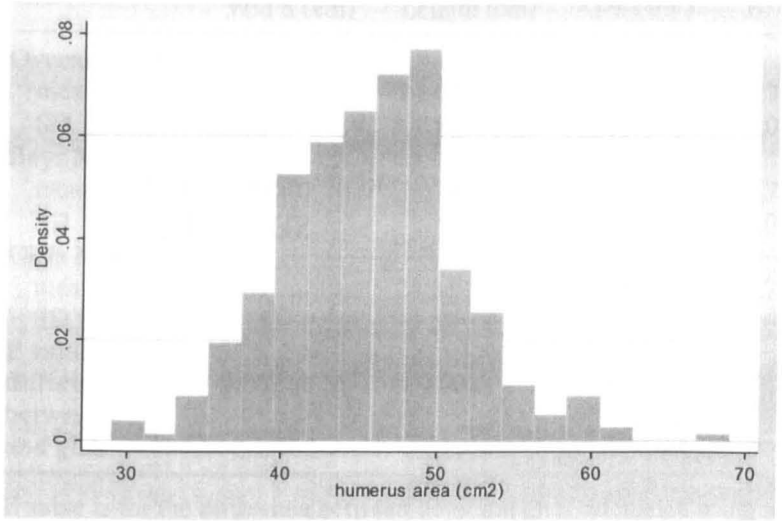
Humerus length is approximately normally distributed:



mean 24.02 cm
SD 1.56

Humerus area

Humerus area is somewhat positively skewed.

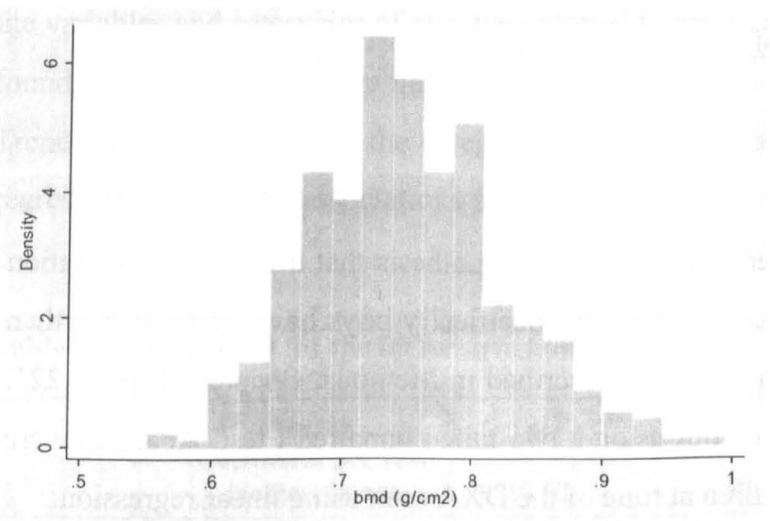


mean 45.61 cm^2
SD 5.73

Because of this non-normal distribution, the median ± interquartile range were also calculated as 45cm (range 42 to 49). The mean and median are similar and the SD is not too large.

Humerus BMD

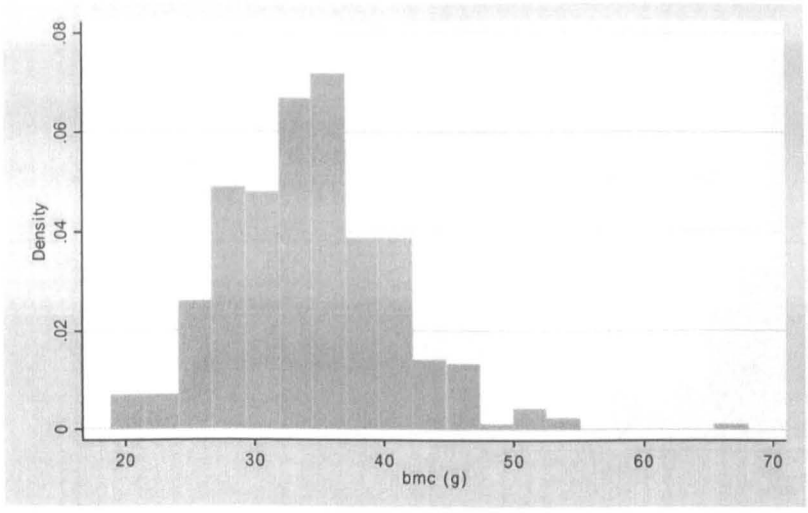
Humerus BMD is approximately normally distributed:



mean 0.749 g/cm2
SD 0.072

Humerus BMC

Humerus BMC is somewhat positively skewed.



mean 34.29g
SD 6.33

Because of this non-normal distribution, the median ± interquartile range were also calculated as 34g (range 30 to 38). The mean and median are similar and the SD is not too large.

Conclusion

Humeral width, length and BMD are approximately normally distributed. However, humeral area and BMC are slightly negatively skewed.

9.5.4.2. Assessing the validity of the humeral variables

Association with gender

To test the validity of the humeral measures, the hypothesis that there is an association between the geometric measures and gender, specifically boys have larger bones than girls, was investigated. Data on gender is described in Methods, Chapter 11, page 227. Unadjusted analyses were carried out using a two-tailed unpaired t test. Analyses were then adjusted for age of the children at time of the DXA scan using linear regression.

See Table 34 on the next page for a summary. In unadjusted analyses, boys had greater humeral width and area, but girls had a greater humeral length. Minor changes were seen in the magnitude of the variables after adjusting for age, but there were no differences seen in direction of difference between the genders.

Table 34: A summary of the mean and SD for each humeral variable for the whole group together, and for boys and girls separately. Results are unadjusted.

	Width (cm)	Length (cm)	Area (cm^2)	BMD (g/cm^2)	BMC (g)
Overall N=389					
mean	1.90	24.02	45.61	0.749	34.29
SD	0.17	1.56	5.73	0.072	6.33
Boys N=196					
mean	1.92	23.74	45.71	0.754	34.52
SD	0.18	1.55	5.83	0.071	6.24
Girls N=193					
mean	1.87	24.31	45.51	0.744	34.06
SD	0.15	1.52	5.64	0.074	6.42
P value for difference between boys and girls	0.002	< 0.001	0.736	0.199	0.481

P value is for the difference between boys and girls calculated using a 2-tailed unpaired t test

Abbreviations: BMC bone mineral content; BMD bone mineral density; cm centimetre; g gram; SD standard deviation

Association with age

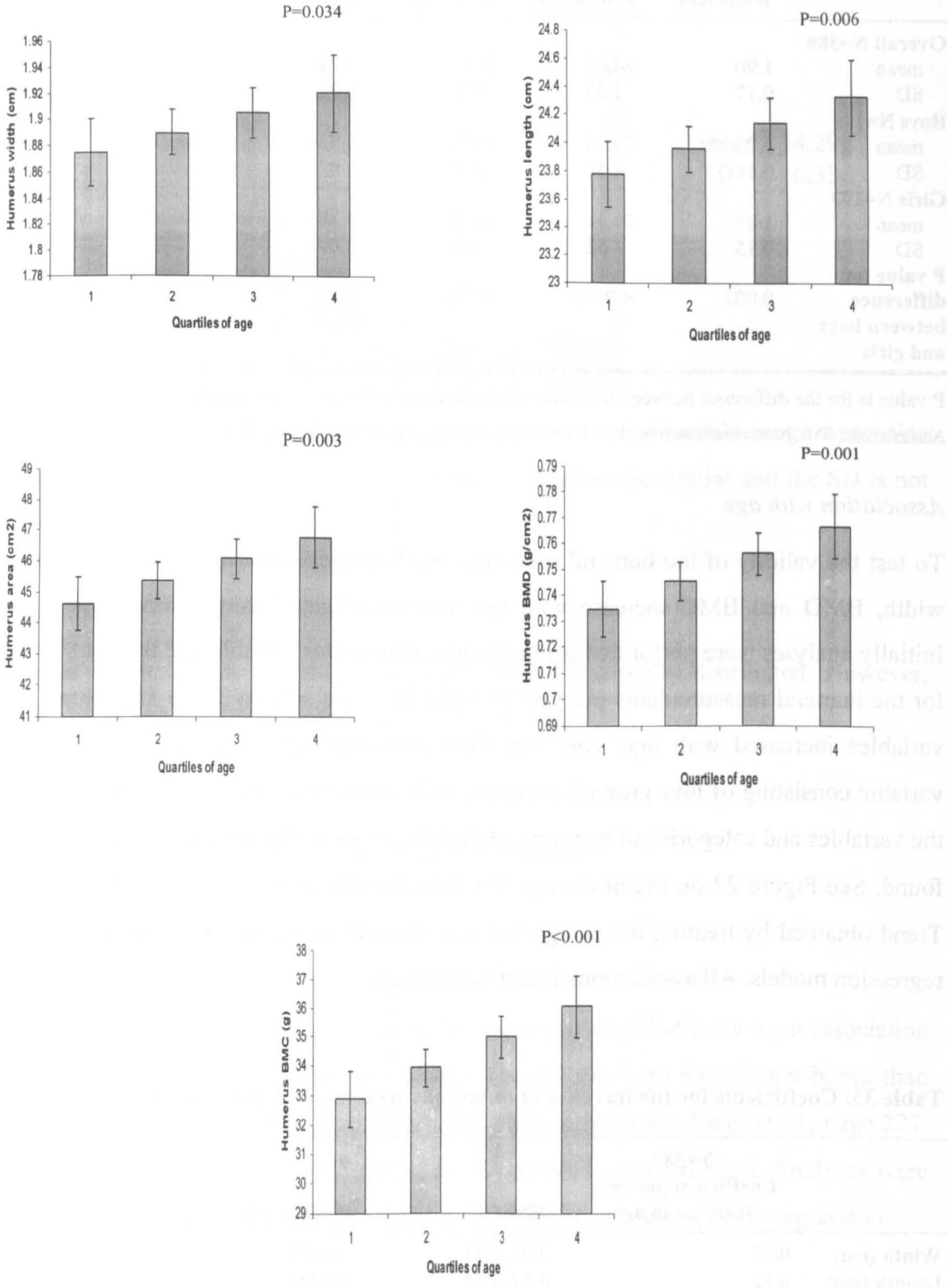
To test the validity of the humeral measures, the hypothesis that humeral area, length, width, BMD and BMC increase with age was investigated using linear regression. Initially analyses were performed using age as a continuous variable and the coefficient for the humeral measurements per year increase in age is seen in Table 35, below. All variables increased with age. Age was then converted into quartiles (a categorical variable consisting of four groups) using the 'xtile' command, and associations between the variables and categories of age were plotted to assess the linearity of any association found. See Figure 27 on the next page. P values for this association were the Test for Trend obtained by treating the categorical age variable as a continuous variable in the regression models. All associations found were linear.

Table 35: Coefficients for the increase in humeral variables, seen per year increase in age

	N=389 Coefficient per year increase in age	95% CI	P value
Width (cm)	0.07	0.02, 0.11	0.005
Length (cm)	0.82	0.39, 1.25	<0.001
Area (cm^2)	3.26	1.69, 4.83	<0.001
BMD (g/cm^2)	0.041	0.021, 0.061	<0.001
BMC (g)	4.47	2.76, 6.18	<0.001

Abbreviations: BMC bone mineral content; BMD bone mineral density; CI confidence interval; cm centimetre; g gram

Figure 27: Humerus variables plotted according to quartiles of age. P values are Test for trend



Abbreviations: BMC bone mineral content; BMD bone mineral density; cm centimeter; g gram

Association with anthropometry

To test the validity of the humeral measures, the hypothesis that there is an association between the humeral measurements and measures of body size such as height and weight was investigated using linear regression. See Methods, Chapter 11, page 242 for a further description of the height and weight variables used. Initially analyses were performed unadjusted using height and weight as continuous variables. Analyses were then repeated adjusting for age and the adjusted coefficient for the humeral measurements per 10 cm increase in height and per 10 kg increase in weight is shown in Table 36, below. All variables increased with increasing height and weight. Minor changes were seen in the size of the coefficients after adjusting for age, but there were no differences seen in direction of the associations. Height and weight were then converted into quartiles using the 'xtile' command, and associations between the variables and categories of height and weight were plotted to assess the linearity of any association found in a similar manner to before (actual graphs not shown). All associations found were linear.

Table 36: Coefficients for the increase in humeral variables, seen per 10 cm increase in height and per 10 kg increase in weight

	Coefficient per 10 cm increase in height (95%CI) N=389	P value	Coefficient per 10 kg increase in weight (95%CI) N=389	P value
Width (cm)	0.13 (0.11, 0.16)	P<0.001	0.11 (0.09, 0.13)	P<0.001
Length (cm)	1.77 (1.60, 1.94)	P<0.001	0.88 (0.68, 1.08)	P<0.001
Area (cm^2)	6.47 (5.84, 7.10)	P<0.001	4.36 (3.68, 5.04)	P<0.001
BMD (g/cm^2)	0.045 (0.034, 0.055)	P<0.001	0.056 (0.048, 0.065)	P<0.001
BMC (g)	6.83 (6.12, 7.54)	P<0.001	5.89 (5.23, 6.54)	P<0.001

Abbreviations: BMC bone mineral content; BMD bone mineral density; cm centimetre; CI confidence interval; g gram

Association with total body DXA variables

To test the validity of the humeral measures, the hypothesis that there is an association between humeral BMD and BMC and total body BMD and BMC was investigated using linear regression. See Methods, Chapter 11 page 239 for a further description of the total body DXA measurements. Children with missing data were excluded from analysis. Initially analyses were performed unadjusted using total body BMD and total body BMC as continuous variables. Analyses were then repeated adjusting for age and the adjusted coefficient for the humeral measurements per 1 g/cm^2 increase in total body

BMD and per 1 g increase in total body BMC is shown in Table 37 below. Humeral BMD and BMC were positively associated with total body BMD and BMC. Total body BMD and BMC were then converted into quartiles using the 'xtile' command, and associations between the humeral variables and categories of total body BMD or BMC were plotted to assess the linearity of any association found in a similar manner to before (actual graphs not shown). All associations found were linear. The correlation coefficient for the association between humeral BMD and total body BMD was 0.84. The correlation coefficient for the association between humeral BMC and total body BMC was 0.88.

Table 37: Coefficients for the increase in humeral BMD and BMC, seen per 1g/cm^2 increase in total body BMD and per 1g increase in total body BMC

	Coefficient per 1 g/cm^2 increase in total body BMD (95%CI) P value N=384	Coefficient per 1 g increase in total body BMC (95%CI) P value N=384
BMD (g/cm^2)	1.07 (1.00, 1.14) P<0.001	0.0003 (0.00025, 0.00031) P<0.001
BMC (g)	87.3 (80.4, 94.2) P<0.001	0.03 (0.028, 0.031) P<0.001

Abbreviations: BMC bone mineral content; BMD bone mineral density; cm centimetre; CI confidence interval; g gram

Association with pubertal stage

To test the validity of the humeral measures, the hypothesis that there is an association between the humeral measurements and pubertal stage was investigated using linear regression. Pubertal measurements used were girls breast development and boys pubic hair development (see Methods, Chapter 11, page 237 for a further description). Children with missing data were excluded from analysis. Initially analyses were performed unadjusted separately for boys and girls. Analyses were then repeated adjusting for age and the adjusted coefficient for the humeral measurements per increase in pubertal stage is shown in Table 38 on the next page. All variables increased with increasing puberty in girls. No association was seen between humeral measurements and pubertal stage in boys. Minor changes were seen in the size of the coefficients after adjusting for age, but there were no differences seen in direction of the associations. Associations between the variables and pubertal stage were plotted to assess the linearity of any association found in a similar manner to before (actual graphs not shown). All associations found were linear.

Table 38: Coefficients for the increase in humeral width, length, area, BMD and BMC, see per Tanner stage increase in puberty

	GIRLS N=142		BOYS N=132	
	Coefficient per Tanner breast development stage increase in puberty (95%CI)	P value	Coefficient per Tanner pubic hair development stage increase in puberty (95%CI)	P value
Width (cm)	0.04 (0.007, 0.075)	P=0.019	0.02 (-0.08, +0.11)	P=0.743
Length (cm)	0.57 (0.24, 0.90)	P=0.001	0.59 (-0.26, +1.44)	P=0.175
Area (cm^2)	2.14 (0.91, 3.37)	P=0.001	1.53 (-1.55, +4.61)	P=0.327
BMD (g/cm^2)	0.029 (0.012, 0.046)	P=0.001	0.000 (-0.039, +0.040)	P=0.999
BMC (g)	3.01 (1.62, 4.40)	P<0.001	1.18 (-2.14, +4.50)	P=0.484

N=142 for girls and 132 for boys as these are the numbers of children who had humeral measurements and data on reported pubertal stage. See Results, Chapter 11, page 236 for numbers of children with missing pubertal data.

Abbreviations: BMC bone mineral content; BMD bone mineral density; cm centimetre; CI confidence interval; g gram

9.5.4.3. Conclusion of validation study

The measures of humeral geometry (length, width and area) from total body DXA scans using the developed method have face validity i.e. are associated with what is predicted, but this doesn't mean necessarily that they represent true humeral geometry well. As expected, all the humeral measures increased with age and were positively associated with height and weight. Humeral BMD and BMC correlated well with total body BMD and BMC. In girls, as expected, all humeral measures also increased with increasing pubertal stage. There was no association with pubertal stage in boys, perhaps reflecting the poor validity of the pubic hair development variable as a measure of pubertal status, rather than poor validity of the humeral measures.

9.5.5. Conclusion

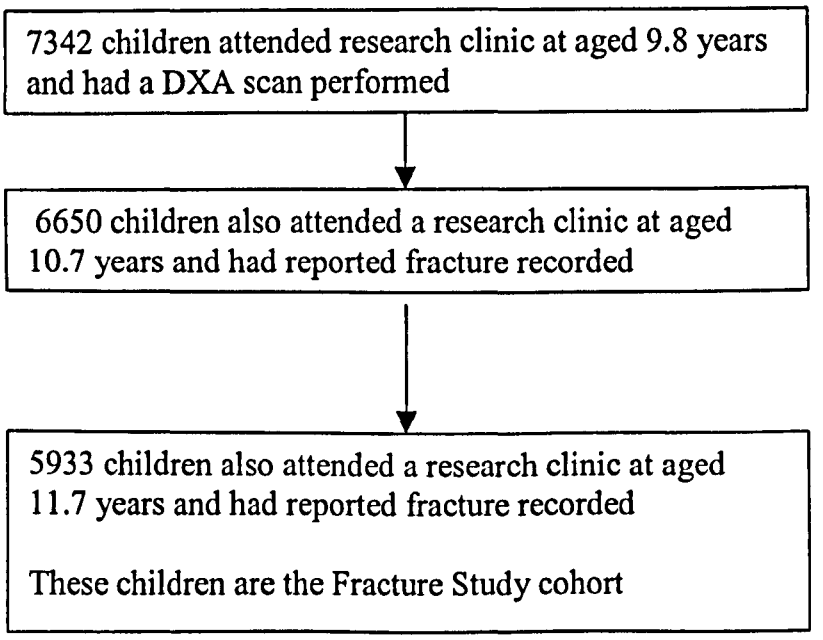
The novel method of producing measures of humeral geometry described above produces both precise and valid estimates of humeral width, length, area, BMD and BMC.

9.6. ASCERTAINMENT AND VERIFICATION OF FRACTURES

9.6.1. Ascertainment of reported fractures

At research clinics approximately 12 and 24 months after their DXA scan, children were asked by the clinic staff if they had fractured a bone since their DXA scan. This was recorded by ticking on a form either a box marked 'yes' or a box marked 'no'. This measure of reported fracture was used as the outcome for this Fracture Study. Only children who attended both research clinics 12 and 24 months since their DXA scan were included (see Figure 28 below). Forms were mechanically scanned and coded, and a database was produced. This database was linked with data previously collected as part of the main ALSPAC study (see Methods, Chapter 8, Table 32, page 196) to allow statistical analysis.

Figure 28: Flow diagram showing children who attended both research clinics 12 and 24 months after their DXA scan at aged 9.8 years



Abbreviations: DXA dual energy X-ray absorptiometry

9.6.2. Verification of fractures

Verification of the fractures comprised three processes: designing the information leaflet and consent forms; piloting the practicalities of obtaining the reports; and carrying out the process on all children who reported fractures.

9.6.2.1. Designing the information leaflet and consent forms

To obtain copies of X-ray reports it was decided to send each child who reported a fracture and their parents an envelope containing a covering letter (Appendix D, page 420), an information leaflet (Appendix E, page 421), a consent form (Appendix F, page 422) and a stamped addressed envelope for return of the completed consent form. Children/parents who did not reply within four weeks would be sent a letter to remind them (Appendix G, page 423). I designed all of these components. The information leaflet was modified in response to comments from the Ethics Committees.

The information leaflet

The original version of the information leaflet is shown in Appendix E, page 421. The Ethics Committee wanted further information included, specifically

- an introductory paragraph
- information on why the study was being done
- information on what factors were being looked at
- information on what implications there were for the children concerned including risks or benefits
- information on confidentiality
- information that participation was voluntary
- information on how X-ray data were to be linked to their ALSPAC data
- information on 'What if something goes wrong?'
- my name, departmental address and contact details clearly displayed on official ALSPAC headed notepaper

The final approved version of the information leaflet is shown in Appendix H, page 424.

9.6.2.2. Piloting the practicalities of obtaining the X-ray reports

To pilot the practicalities of obtaining a copy of the X-ray report once I had the completed consent form, I attended one of the local Paediatric Fracture Clinics run by Consultant Orthopaedic Surgeon Mr Martin Gargan at the Bristol Royal Infirmary (BRI). At the clinic, consent was obtained from 6 parents and assent from their children, to obtain a copy of the X-ray report for their recent fracture. These consent forms were photocopied and sent to the Superintendent Radiographer at the BRI, Mrs Sally King. She then printed copies of the reports and sent them to me through the post. Any questions or queries were dealt with by email or over the telephone.

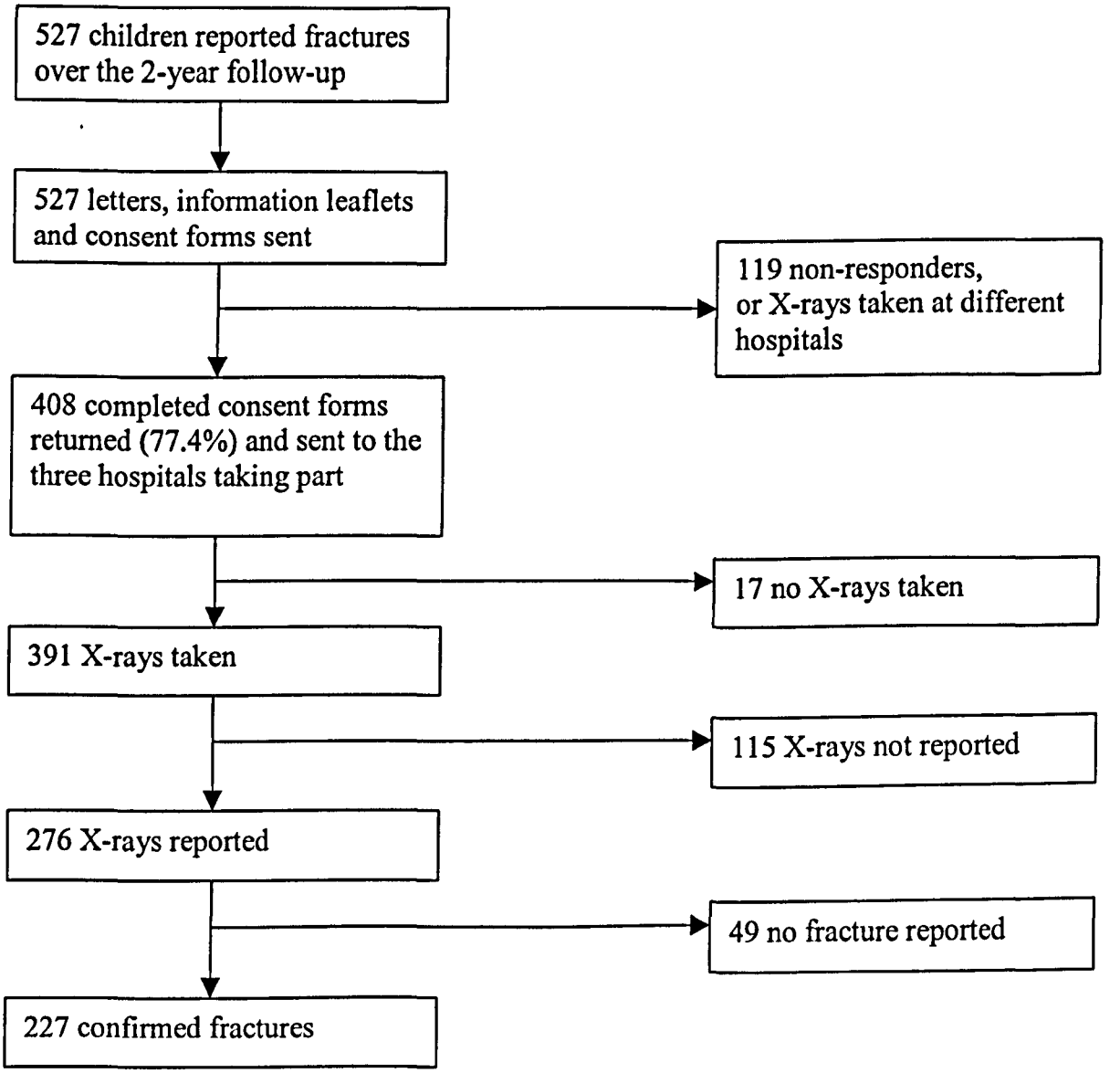
9.6.2.3. Verification of all fractures

The names and addresses of children and their parents who indicated they had fractured a bone since their DXA scan were collated by the Family Liaison Team from ALSPAC. The Family Liaison Team then sent me batches of 50 names and addresses, and they were sent the letter containing the information leaflet (Appendix H, page 424) and consent form (Appendix F, page 422) to allow us to obtain copies of their X-ray reports. The children were divided into batches of 50 so that checks could be made to ensure individual families were not being overloaded with multiple different letters or questionnaires from other researchers using ALSPAC.

Once completed consent forms had been returned, these were sent to named personnel within the Radiology Departments of three local hospitals. X-ray reports, where available, were printed out and sent to me through the post. Data were then extracted on whether or not a fracture had been identified. Approval (ethical and local trust / research governance) had been obtained from three local hospitals (United Bristol Healthcare Trust, North Bristol and Weston) for this process. Also the Caldicott Guardians at each hospital had given their approval to obtain copies of X-ray reports for research purposes.

In total, 527 consent forms were sent out, and X-ray reports were obtained in 276 (52.4%). Of these, 227 (82.3%) confirmed a fracture. See Figure 29 on the next page for a flow diagram.

Figure 29: Flow diagram showing verification of reported fractures



CHAPTER 10: GENERAL STATISTICAL METHODS

The previous two methods chapters have covered the general methods of ALSPAC and the specific methods of the Fracture Study. This chapter describes the general statistical methods used. More detailed specific statistical methods are included in each of the results chapters. Although ALSPAC is a cohort study, no repeated measures have been used for the Fracture Study. Participants with missing data were excluded from some analyses.

10.1. STATISTICAL PACKAGE

All statistical analyses were performed using Stata statistical software version 8 (Stata Corp, College Station, Texas).

10.2. POWER CALCULATION

Before starting this study a power calculation was performed using OpenEpi, an open source website containing statistical programmes for Public Health (www.openepi.com). This uses the following equation derived from Fleiss, 1981 (491).

$$Power = \Phi\left(\frac{\sqrt{(n_1 * \Delta^2)} - z_{1-\alpha/2}\sqrt{(1+1/\kappa) * p * q}}{\sqrt{(p_1 * q_1) + (p_2 * q_2 / \kappa)}} \right)$$

where

Δ = difference of risk of disease between exposed group and non-exposed group;

κ = ratio of sample size: non-exposed group / exposed group;

p_1 = risk of disease among exposed group;

p_2= risk of disease among non-exposed group;

$p = (p_1 * n_1 + p_2 * n_2) / (n_1 + n_2)$;

$q = 1-p$;

n_1= available sample size among exposed group;

$RR = (p_1/p_2)$.

For the power calculation it was assumed that 7000 children would be followed up for 2 years and that approximately 2% of the children would fracture each year (see Literature Review, Chapter 5, page 122). Assuming a 95% significance level (i.e. P<0.05), power was calculated for a range of relative risks for fracture comparing children with a BMD Z score of less than -1 children with a BMD Z score of greater than -1. See Table 39 below.

Table 39: Power calculation for the Fracture Study with significance set at p<0.05 to detect a range of differences in fracture risk between those children with a BMD Z score less than -1 compared to those children with a BMD Z score greater than -1

	'Exposed' i.e. BMD <-1 N=2240	'Unexposed' i.e. BMD >-1 N=11760	Relative risk of fracture	Power
Risk of fracture	2.6%	2%	1.3	44%
Risk of fracture	2.8%	2%	1.4	67%
Risk of fracture	3.0%	2%	1.5	85%
Risk of fracture	3.2%	2%	1.6	95%

Abbreviations: BMD bone mineral density

So, if the above assumptions were true there would be a 90% power to detect a 55% increase in relative risk of fracture in children with a BMD Z score less than 1. This compares favourably with previous estimates for relative risk of radial fracture in children with a low BMD (451).

10.3. CONVERTING CONTINUOUS VARIABLES INTO CATEGORICAL VARIABLES

To convert continuous variables to categorical variables various methods were used. For categories based on standard definitions such as birth weight (see Methods, Chapter 8, page 185) divided into low (less than 2500g) or normal (≥ 2500g) the '*recode*' function was used:

e.g. *recode birthweight min/2500=1 2500/max=2*

Gestational age was converted to categories in a similar manner. Other variables such as locomotor ability were converted to categories based on percentages such as tertiles using the '*xtile*' command:

e.g. *xtile locomotorability=locomotorability, n(3)*

Other variables converted to categories in a similar manner were balance, dietary data, total body fat and lean mass, child height, child weight, maternal body size, maternal age and paternal body size. Where variables were not normally distributed such as fat mass (see page 241), variables were transformed to an approximate normal distribution before being converted into categories based on percentages.

10.3.1. Z scores

In some analyses the DXA measurements were converted to Z scores, which have a mean of 0 and a SD of 1. This was done by subtracting from the mean and dividing by the standard deviation. Coefficients produced by regression are therefore per SD change. The value of using Z scores is that comparisons can be made across populations and against reference curves in order to improve their interpretability (492).

10.4. DISTRIBUTIONS, DATA CHECKING AND SUMMARY STATISTICS

To assess distributions of continuous variables, histograms were drawn using the '*hist, freq norm*' command and means and standard deviations produced if normally distributed using the '*summ, detail*' command. Frequency tables were produced for categorical variables using the '*tab*' command.

10.4.1. Transformations of skewed continuous variables

If a variable was not normally distributed, the mean ± SD of the variable was compared to the median and interquartile range (IQR). If there was a potentially important difference between the mean and median (arbitrarily chosen to be more than 3% difference) or the SD was considered large, then the variable was transformed using the '*gladder*' command to find the best approximation of a normal distribution. Transformation of non-normally distributed data can be necessary because with skewed data the SD in different groups may be very different, or the relationship between the outcome and exposure variables may not be linear, violating the assumptions of linear regression methods (492).

10.4.2. Data checking

Although the data from ALSPAC had already been cleaned, minimum and maximum values were assessed for plausibility. Range checks were performed to look for extreme values. Values of DXA measurements of bone mass were compared with other populations published in the literature (see Literature Review, Chapter 3, page 54), and the values were found to be similar.

10.5. STATISTICAL ASSOCIATIONS

10.5.1. Between two continuous variables

Scatter plots were used to display the data using the '*scatter*' command. Regression lines were fitted to the scatter plots to test for an association. Pearson's correlation coefficient was calculated using the '*correlate*' command if the distributions were normal.

10.5.2. Between a categorical and a continuous variable

Bar charts were used to display the data. For a binary outcome e.g. fractures, a two-tailed unpaired t test was used to test for an association using the '*ttest*' command. For a categorical outcome such as social class the analysis of variance (ANOVA) test was used to assess for an association using the '*oneway*' command.

10.5.3. Between two categorical variables

Cross-tabulations were used to present the data. Chi-squared test was used to assess if there was an association using the '*chi*' command. The Mantel-Haenszel technique was used to estimate the magnitude of the association using the '*mhodds*' command to produce crude odds ratios (OR), where the null hypothesis was that there was no difference between the two groups.

10.5.4. Test for linear trend

To investigate if an exposure effect is linear, it is assumed that the outcome increases or decreases systematically with the exposure effect. For continuous variables a scatter plot was drawn to exclude non-linear associations such as quadratic or U-shaped associations. In linear regression models with continuous outcomes such as bone mass, the linear effect corresponds to a constant increase in the mean of the outcome per unit increase in the exposure variable. A test for trend in this situation is an approximation to

a likelihood ratio test of the null hypothesis that the regression coefficient for a linear effect is zero.

In logistic regression models with a binary variable such as fractures as the outcome, the linear effect corresponds to a constant increase in the log odds per unit increase in the exposure variable. P values in this situation will be the test for trend, calculated by treating the categorical variable as continuous in the regression analysis.

The existence of a linear trend or dose response relationship may provide more convincing evidence of a causal effect of an exposure than a simple comparison of exposed with unexposed subjects (492).

10.5.5. P values and significance

In the Fracture Study all P values are given to three decimal places. The P values are interpreted on a continuous scale without any arbitrary cut-off defining significant or non-significant (493). It is assumed that the smaller the P value, the stronger the evidence against the null hypothesis. Where multiple comparisons have been made, the smallest P value possible ($P<0.001$) is taken as reasonable evidence against the null hypothesis.

10.5.6. Missing data approaches

In the Fracture Study, participants with missing data have been compared to participants without missing data to assess differential loss, or selection, which may bias the results. During statistical analysis of associations, participants with missing data have been excluded from analysis. Results have then been interpreted in light of any potential bias. It is recognised that there are statistical imputational methods available to approach missing data such as unweighted or weighted average of ordinary least squares, conditional linear model estimates or random effects estimates (494), but they have not been employed in the Fracture Study.

10.6. ADJUSTING FOR POTENTIAL CONFOUNDERS

10.6.1. Mantel-Haenszel method

To adjust for the potential confounding effects of one other variable, the potential confounding variable was assessed to see if it was associated with both the outcome variable and the explanatory variable by the above methods. The outcome variable was stratified according to the potential confounder and the odds for each strata were calculated. The adjusted odds ratio was then calculated using the '*mhodds*' command.

10.6.2. Regression techniques

The potential confounding variable was assessed to see if it was associated with both the outcome variable and the explanatory variable by the above methods. With a binary outcome e.g. fractures, multivariable logistic regression was used to calculate odds ratios with 95% confidence intervals (CIs) for associations adjusted for measured potential confounders using the '*logistic*' command. For continuous outcomes such as bone area, multivariable linear regression was used to calculate adjusted regression coefficients with 95% CIs using the '*regress*' command. P values for multivariable linear regression were calculated using the Z test. For categorical outcomes such as social class multivariable linear regression was used to calculate adjusted regression coefficients with 95% confidence intervals. P values in this situation were the test for trend, calculated by treating the categorical variable as continuous in the regression analysis. Variables were sequentially added to the regression equations. R^2 values presented are the adjusted-R^2 and represent the proportion of variability in the dependent variable explained by the statistical model.

10.6.3. Choice of potential confounders

The choice of confounders was based on the literature review, and also their suspected place in the causal pathway. Variables were not adjusted for if they were considered to be intermediate variables on the causal pathway.

10.6.4. Testing for effect modification or interaction

Stratified analyses were carried out to look for evidence of effect modification or interaction. If there was a pre-specified hypothesis indicating an interaction between two variables was likely, this was further assessed by including a multiplicative interaction term in the regression models and calculating the Likelihood Ratio Test (LRT), using the *'lrtest'* command.

10.7. SUMMARY

Stata 8.0 was used for all statistical analyses. Data were reduced to categorical variables where necessary (i.e. for graphical displays), and data checking was carried out. Various methods were used to investigate associations between variables and to control for potential confounders. More detailed specific statistical methods are included in each of the following results chapters.

CHAPTER 11: DESCRIPTIONS OF THE VARIABLES USED

There are four results chapters. This is the first and describes the variables used in the Fracture Study. The second (Chapter 12) results chapter describes the confounding structure of bone mass. The third (Chapter 13) results chapter describes the associations between child early life factors, dietary data, physical activity, maternal, paternal and socio-economic data with fractures between ages 9.8 and 11.7 years. The fourth (Chapter 14) results chapter describes the association between bone mass measured at aged 9.8 years with fracture risk over the following two years.

11.1. CHILD DATA

A summary of the child data used in the Fracture Study has been shown in tabular form on page 196. The statistical methods used to describe the child data are described starting on page 222. The Fracture Study consisted of 5933 children (see Methods, Chapter 8, Figure 24, page 181).

11.1.1. Gender

The method of data collection for this variable has been described in Methods, Chapter 8, page 184. Of the 5933 children in the Fracture Study, data on gender were available on all. 2901 (48.9%) of the study population were male.

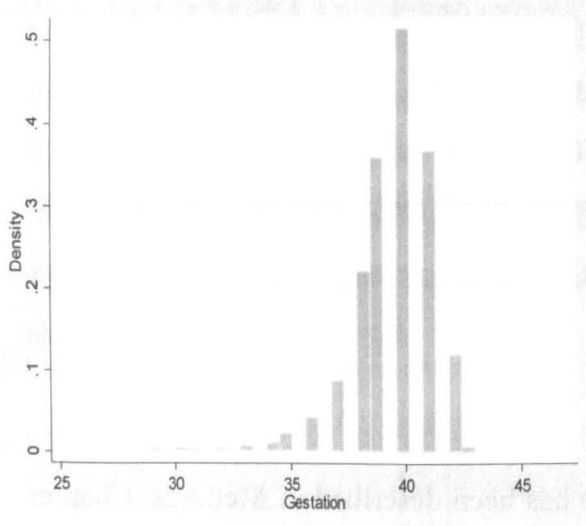
11.1.2. Ethnicity

The method of data collection for this variable has been described in Methods, Chapter 8, page 184. Of the 5933 children in the Fracture Study, data on ethnicity were available on 5410 (91.2%). Of the 5410, 191 (3.5%) were non-white.

11.1.3. Gestational age

The method of data collection for this variable has been described in Methods, Chapter 8, page 184. Of the 5933 children in the Fracture Study, data on gestational age were available on 5645 (95.1%). As expected, gestational age was negatively skewed (see Figure 30 below). The median gestational age was 40 weeks. The interquartile (IQR) range was 39 weeks to 41 weeks. Mean ± SD for gestational age was 39.4 ± 1.9 weeks. As the mean was only approximately 1.5% lower than the median, and the SD was not large, a transformation was not considered necessary (see Methods, Chapter 10, page 222). Of the 5645 children with data available, 316 (5.6%) were classified as pre-term i.e. born before 37 weeks gestation. Consistent with the literature, more boys were born preterm than girls (6.6% vs 4.6%, P=0.001).

Figure 30: Graph showing the distribution of gestational age at delivery of children in the Fracture study.

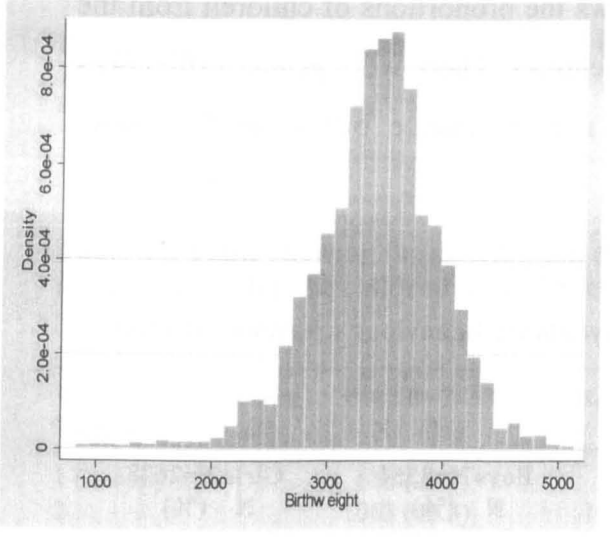


Median 40 weeks
IQR 39 to 41 weeks

11.1.4. Birth weight

The method of data collection for this variable has been described in Methods, Chapter 8, page 185. Of the 5933 children in the Fracture Study, data on birth weight were available on 5578 (94.0%). Birth weight was approximately normally distributed (see Figure 31 on next page). The mean ± SD for birth weight was 3413g ± 549g. Of the 5578 children with data available, 271 (4.9%) were classified as of low birth weight (<2500g). There was no gender difference in the percentage born as low birth weight.

Figure 31: Graph showing the distribution of birth weight (g) of children in the Fracture study.



Mean 3413 g
SD 549 g

11.1.5. Breast feeding

The method of data collection for this variable has been described in Methods, Chapter 8, page 185. Of the 5933 children in the Fracture Study, data on breast feeding were available on 5263 (88.7%). Table 40 below shows the proportions of children from the Fracture Study breast fed. There were no gender differences in breast feeding, but, as expected, there was strong social patterning (e.g. percentage of children never breast fed with mums with degrees = 3.1% versus 35.9% with mums with no formal qualifications or CSEs only, P<0.001).

Table 40: Table showing distribution of breast feeding for children in the Fracture study.

	All N=5263 N (%)
Never breastfed	923 (17.5)
< 1 month	821 (15.6)
1-3 months	830 (15.8)
3-6 months	755 (14.4)
> 6 months	1934 (36.8)

11.1.6. Risk avoidance at aged 3.5 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 185. Of the 5933 children in the Fracture Study, data on risk avoidance were available on 5182 (87.3%). Table 41 below shows the proportions of children from the Fracture Study in the various risk avoidance categories. There were gender differences in risk avoidance, with girls showing more risk avoidance behaviour than boys (P<0.001).

Table 41: Table showing the distribution of risk avoidance behaviour measured at aged 3.5 years for children in the Fracture Study

Risk avoidance behaviour	All N=5182 N (%)	Boys N=2554 N (%)	Girls N=2628 N (%)
Never or hardly ever avoids risks	2238 (43.2)	1220 (47.8)	1018 (38.7)
Sometimes	2132 (41.4)	1002 (39.2)	1130 (43.0)
Often or very often avoids risks	812 (15.7)	332 (13.0)	480 (18.3)

11.1.7. Physical activity

11.1.7.1. Locomotor ability at aged 7.6 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 186. Of the 5933 children in the Fracture Study, data on locomotor ability were available on 4986 (84.0%). Girls had a more advanced locomotor ability than boys at aged 7.6 years (P<0.001). See Table 42 below.

Table 42: Table showing the distribution of locomotor ability measured at aged 7.6 years separately for boys and girls in the Fracture Study

Tertiles of locomotor ability	Boys N=2452 N (%)	Girls N=2534 N (%)
1 (lowest)	1207 (49.2)	905 (35.7)
2	662 (27.0)	734 (29.0)
3 (highest)	583 (23.8)	895 (35.3)

11.1.7.2. Time spent watching television per week at aged 4.5 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on time spent watching TV were available on 4969 (83.8%). Boys watched more television than girls, but the difference was only modest (13.0 hours vs 12.5 hours per week, P=0.011). See Table 43 on next page.

Table 43: Table showing the distribution of time spent watching television per week at aged 4.5 years for the children in the Fracture Study

Time spent watching TV per week	Boys N=2453 N (%)	Girls N=2516 N (%)
1 (lowest)	630 (25.7)	742 (29.5)
2	1063 (43.3)	1043 (41.5)
3 (highest)	760 (31.0)	731 (29.1)

11.1.7.3. Time spent in a vehicle per week at aged 4.5 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on time spent in a vehicle were available on 4958 (83.6%). Girls spent more time in a vehicle per week than boys, but again the difference was modest (4.7 hours vs 4.5 hours per week, P=0.018). See Table 44 below.

Table 44: Table showing the distribution of time spent in a vehicle per week at aged 4.5 years for children in the Fracture Study

Time spent in vehicle per week	Boys N=2451 N (%)	Girls N=2507 N (%)
1 (lowest)	303 (12.4)	248 (9.9)
2	1330 (54.3)	1418 (56.6)
3 (highest)	818 (33.4)	841 (33.6)

11.1.7.4. Time spent outdoors in summer at aged 4.5 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on time spent outdoors in summer were available on 4964 (83.7%). There was no gender difference in time spent outdoors during summer. 73.0% of children spent more than 28 hours outside per week in the summer.

11.1.7.5. Time spent outdoors in winter at aged 4.5 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on time spent outdoors in winter were available on 4947 (83.4%). There was no gender difference in time spent outdoors during winter. 52.5% of children spent more than 10.5 hours outside per week in the summer.

11.1.7.6. Number of episodes of vigorous physical activity per week at aged 9 years

The method of data collection for this variable has been described in Methods, Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on number of episodes of vigorous physical activity were available on 3580 (60.3%). Mothers of boys report more vigorous activity per week than girls (P<0.001). See Table 45 below.

Table 45: Table showing the distribution of episodes of vigorous physical activity at aged 9 years for the children in the Fracture Study

Episodes of vigorous physical activity per week	Boys N=1737 N (%)	Girls N=1843 N (%)
less than 4	816 (47.0)	1173 (63.7)
4 to 6	547 (31.5)	515 (27.9)
daily	374 (21.5)	155 (8.4)

11.1.8. Balance at aged 7.6 years

11.1.8.1. Number of correct steps in total

The method of data collection for this variable has been described in Methods, Chapter 8, page 186. Of the 5933 children in the Fracture Study, data on number of correct steps in total were available on 4837 (81.5%). Girls took more correct steps in total than boys (P<0.001). See Table 46 below.

Table 46: Table showing the distribution of the number of correct steps in total at aged 7.6 years, performed by the children in the Fracture Study

Number of correct steps in total	Boys N=2387 N (%)	Girls N=2450 N (%)
1 (lowest)	415 (17.4)	242 (9.9)
2	892 (37.4)	779 (31.8)
3 (highest)	1080 (45.3)	1429 (58.3)

11.1.8.2. Number of correct steps before an error

The method of data collection for this variable has been described in Methods, Chapter 8, page 186. Of the 5933 children in the Fracture Study, data on number of correct steps before an error were available on 4837 (81.5%). Girls took more correct steps before an error than boys (P<0.001). See Table 47 below.

Table 47: Table showing the distribution of the number of correct steps before an error at aged 7.6 years, performed by the children in the Fracture Study

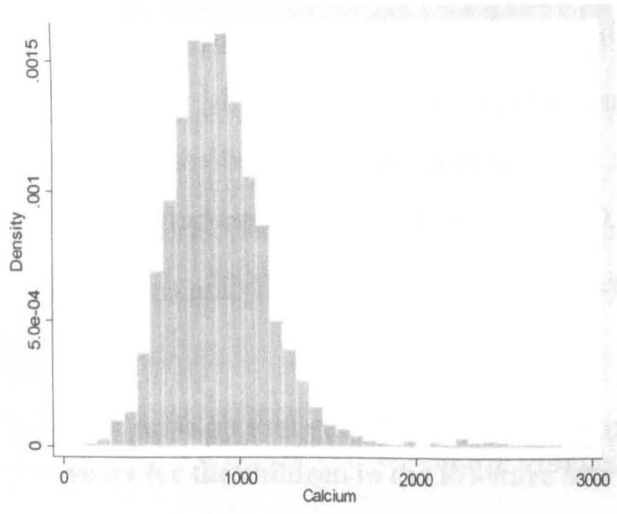
Number of correct steps before an error	Boys N=2387 N (%)	Girls N=2450 N (%)
1 (lowest)	597 (25.0)	440 (18.0)
2	710 (29.7)	581 (23.7)
3 (highest)	1080 (45.3)	1429 (58.3)

11.1.9. Dietary data at aged 6.8 years

11.1.9.1. Calcium intake

The method of data collection for this variable has been described in Methods Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on calcium intake were available on 4946 (83.4%). Calcium intake was positively skewed (see Figure 32 below). The median daily calcium intake was 871mg and the IQR was 711mg to 1041mg. The mean ± SD for daily calcium intake was 900 ± 302mg. As the mean was more than 3% different to the median, and the SD was considered large, a square-root transformation was carried out (see Methods Chapter 10, page 222). Girls had a lower unadjusted calcium intake than boys (P<0.001), but after adjustment for daily energy intake there was no gender difference.

Figure 32: Distribution of daily calcium intake at aged 6.8 years for the children in the Fracture Study



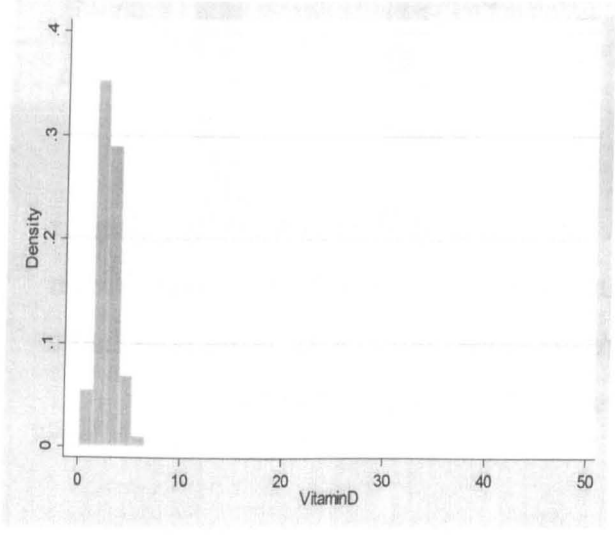
Median 871 g
IQR 711g to 1041g

11.1.9.2. Vitamin D intake

The method of data collection for this variable has been described in Methods Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on vitamin D intake were available on 4946 (83.4%). Vitamin D intake was positively skewed (see Figure 33 on next page). The median daily vitamin D intake was 2.73 μg, and the IQR was 2.16 to 3.40 μg. The mean ± SD for daily vitamin D intake was 2.84 ± 1.16μg. As the mean was more than 3% different to the median, and the SD was considered large, a

logarithmic transformation was carried out (see Methods Chapter 10, page 222). Girls had a lower unadjusted vitamin D intake than boys (P=0.015), but after adjustment for daily energy intake there was no gender difference seen.

Figure 33: Distribution of daily vitamin D intake at aged 6.8 years for the children in the Fracture Study

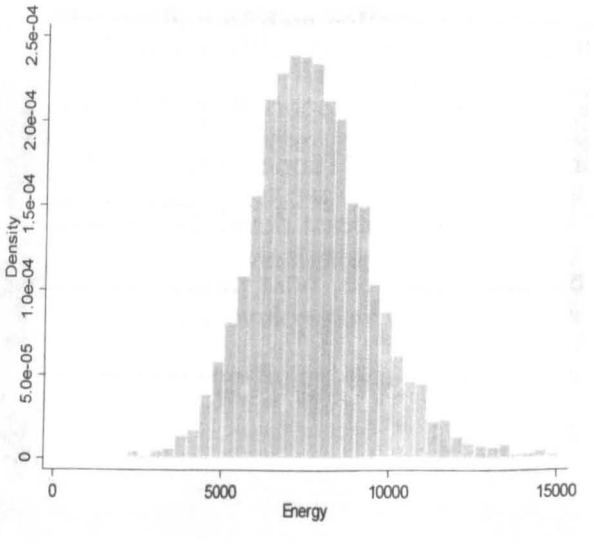


Median 2.73 μg
IQR 2.16 to 3.40 μg

11.1.9.3. Total energy intake

The method of data collection for this variable has been described in Methods, Chapter 8, page 187. Of the 5933 children in the Fracture Study, data on total energy intake were available on 4946 (83.4%). Daily total energy intake was approximately normally distributed (see Figure 34 below) with a mean ± SD of 7705 kj ± 1771 kj. Boys had a greater daily total energy intake than girls (mean ± SD for boys 7866kj ± 1804 and for girls 7549kj ± 1724, P<0.001).

Figure 34: Distribution of total energy intake at aged 6.8 years for children in the Fracture Study



Mean 7705 kj
SD 1771 kj

11.1.10. Psychological status at aged 7.6 years

11.1.10.1. Attention-deficit hyperactivity disorder (ADHD)

The method of data collection for this variable has been described in Methods, Chapter 8, page 188. Of the 5933 children in the Fracture Study, data on ADHD were available on 4978 (83.9%). Of these 4978, 88 (1.8%) were diagnosed with ADHD. More boys had a diagnosis of ADHD than girls (3.0% of boys versus 0.6% of girls, P<0.001).

11.1.10.2. Oppositional/conduct disorder (OCD)

The method of data collection for this variable has been described in Methods, Chapter 8, page 188. Of the 5933 children in the Fracture Study, data on OCD were available on 4978 (83.9%). Of these 4978, 140 (2.8%) were diagnosed with OCD. More boys had a diagnosis of OCD than girls (4.3% of boys versus 1.4% of girls, P<0.001).

11.1.11. Pubertal status at aged 9.6 years

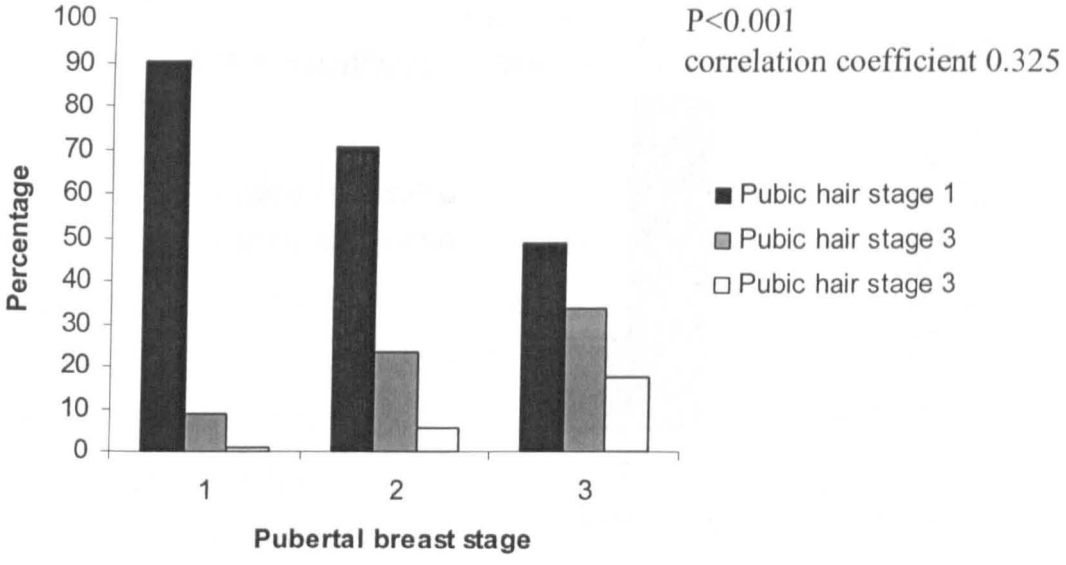
11.1.11.1. Girls breast development

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 3032 girls in the Fracture Study, data on girls breast development were available on 2460 (81.1%). In terms of reported breast development, 63.2% of girls were prepubertal, 30.9% in early puberty (Tanner stage 2) and 5.9% in later puberty.

11.1.11.2. Girls pubic hair development

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 3032 girls in the Fracture Study, data on girls pubic hair development were available on 2471 (81.5%). In terms of reported pubic hair development, 81.9% were prepubertal, 14.7% in early puberty (Tanner stage 2) and 3.4% in later puberty. To investigate agreement between girls breast development and girls pubic hair development a graph was plotted (see Figure 35, next page) and the correlation coefficient was calculated to be 0.325.

Figure 35: Bar chart showing agreement between Tanner pubertal stage of breast development and pubic hair development for girls



P value calculated by Chi-squared test

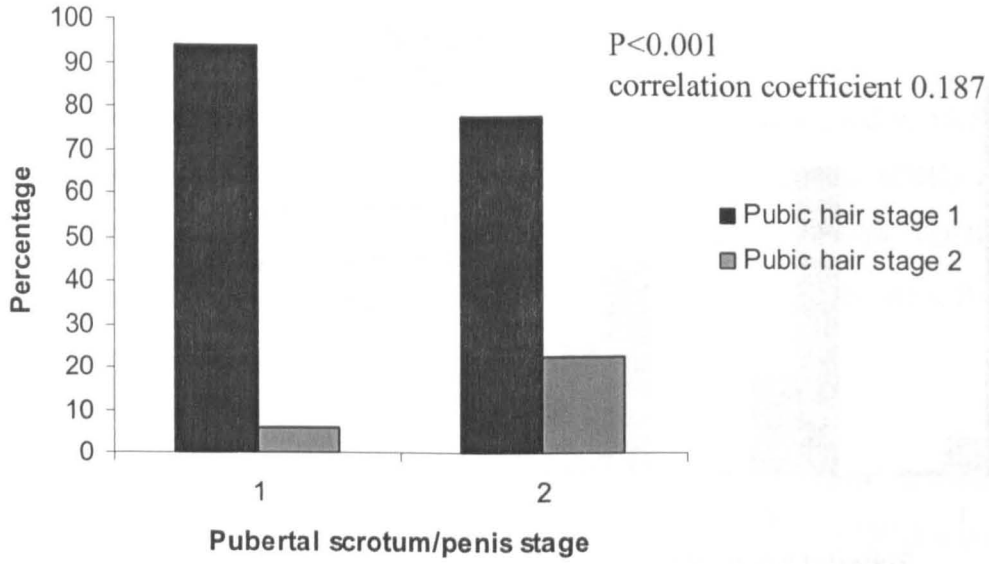
11.1.11.3. Boys scrotum/penis development

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 2901 boys in the Fracture Study, data on boys scrotum/penis development were available on 2088 (72.0%). In terms of reported scrotum/penis development, 26.0% were prepubertal (Tanner stage 1) and 74.0% were in early puberty (all other Tanner stages). This is unlikely, as 63-82% of girls were prepubertal at this age. It seems that reported scrotum/penis development over-estimates the boys pubertal development, and so this variable will not be used in any further analyses.

11.1.11.4. Boys pubic hair development

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 2901 boys in the Fracture Study, data on boys pubic hair development were available on 2038 (70.3%). In terms of reported pubic hair development, 82.2% were prepubertal (Tanner stage 1) and 17.8% were in early puberty. To investigate agreement between boys scrotum/penis development and boys pubic hair development a graph was plotted (see Figure 36, next page) and the correlation coefficient was calculated to be 0.187.

Figure 36: Bar chart showing agreement between Tanner stage of scrotum/penis development and pubic hair development for boys

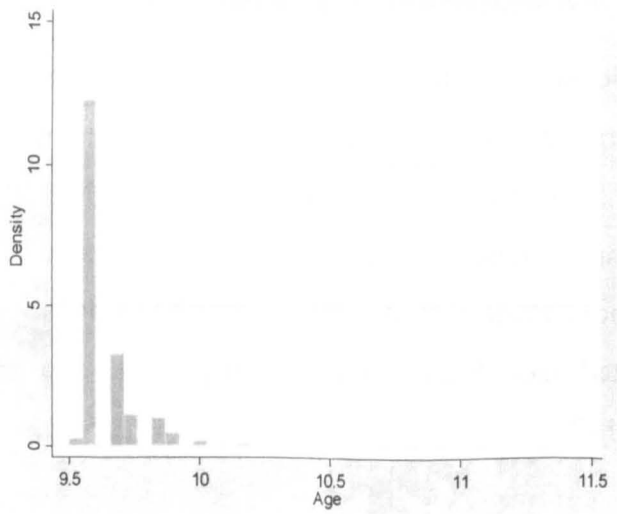


P value calculated by Chi-squared test

11.1.11.5. Age at puberty measurements

The method of data collection for this variable has been described in Methods Chapter 8, starting on page 189. Of the 5933 children in the Fracture Study, data on age at puberty measurements were available on 4712 (79.4%). Age at puberty measures was positively skewed by a few outliers with a median age of 9.6 years and an IQR of 9.6 to 9.7 years. The mean ± SD for age at puberty measurements was 9.6 ± 0.1 years. As the mean was no different to the median and the SD was small, transformation was not considered necessary (see Figure 37 below).

Figure 37: Distribution of age at puberty measurement for the children in the Fracture Study



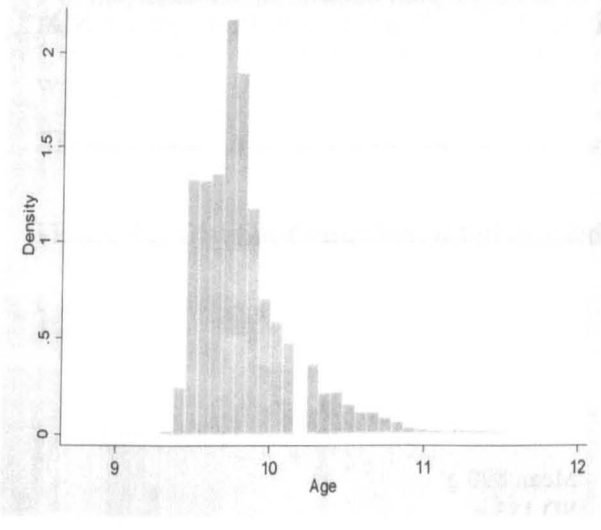
11.1.12. DXA measurements

Presented below are the distributions of the DXA measurements. The confounding structure and association with gender etc is addressed fully in Chapter 12.

11.1.12.1. Age at DXA scan

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 5933 children in the Fracture Study, data on age at DXA scan were available on all. Age was not normally distributed (see Figure 38 below). The median age was 9.8 years with the IQR 9.7 to 10.0 years. The mean ± SD for age at DXA scan measurements was 9.8 ± 0.3 years. As the mean was no different to the median and the SD was small, transformation was not considered necessary. There were no gender differences in age at DXA scan, but it was socially patterned e.g. children whose mothers had degrees were younger compared to those whose mothers had no formal education or CSEs only (P<0.001). Speaking to ALSPAC clinic staff, it seems that the parents of children with higher socio-economic backgrounds book into the research clinics well in advance and these children are seen earlier and so are younger than children from lower socio-economic backgrounds.

Figure 38: Distribution of age (years) at time of DXA scan for the children in the Fracture Study

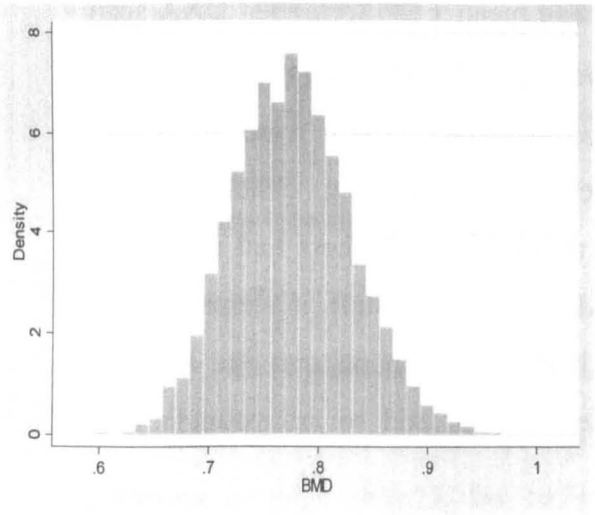


Median 9.8 years
IQR 9.7 to 10.0 years

11.1.12.2. TBLH BMD

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 5933 children in the Fracture Study, data on TBLH BMD were available on all. TBLH BMD was normally distributed with a mean ± SD of 0.777g/cm^2 ± 0.054 (see Figure 39 below).

Figure 39: Distribution of TBLH BMD for the children in the Fracture Study

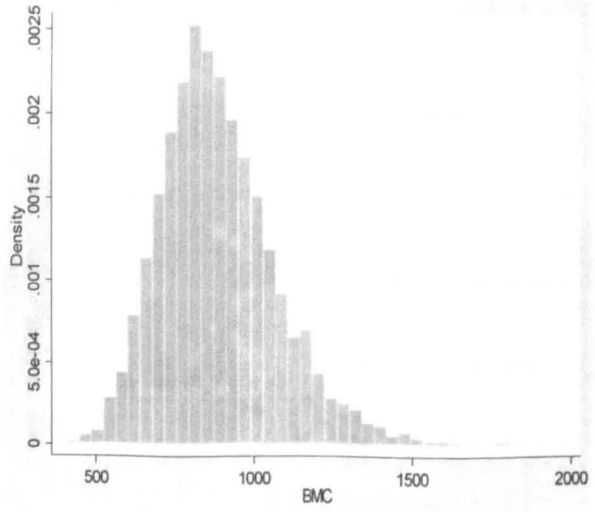


Mean 0.777 g/cm^2
SD 0.054 g/cm^2

11.1.12.3. TBLH BMC

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 5933 children in the Fracture Study, data on TBLH BMC were available on all. TBLH BMC was approximately normally distributed with a mean ± SD of 890g ± 183 (see Figure 40 below).

Figure 40: Distribution of TBLH BMC for the children in the Fracture Study

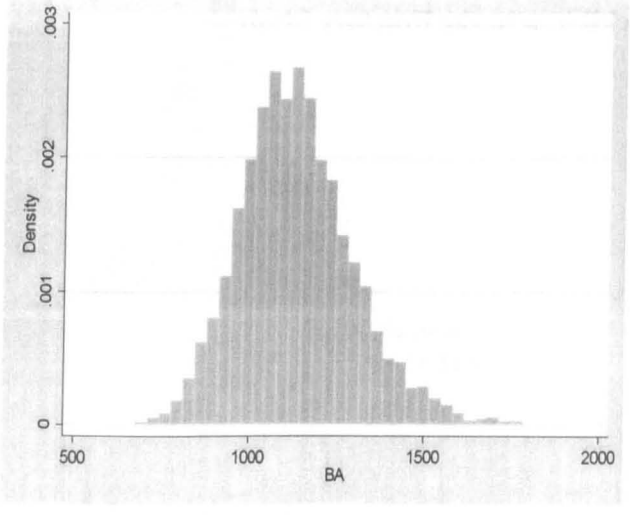


Mean 890 g
SD 183 g

11.1.12.4. TBLH bone area

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 5933 children in the Fracture Study, data on TBLH bone area were available on all. TBLH bone area was approximately normally distributed with a mean ± SD of 1136cm^2 ± 164 (see Figure 41 below).

Figure 41: Distribution of bone area for the children in the Fracture Study

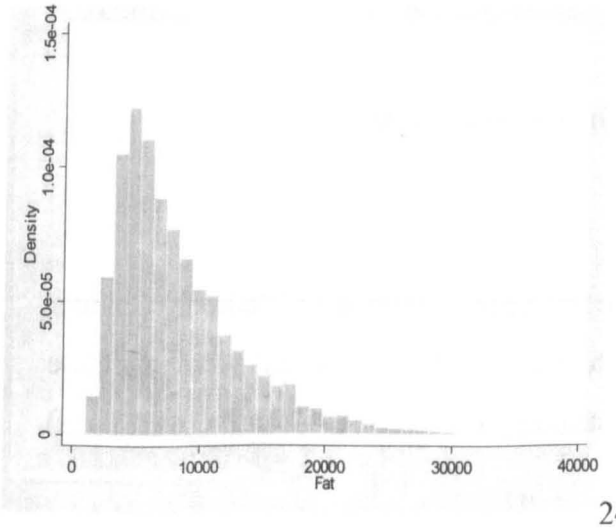


Mean 1136 cm^2
SD 164 cm^2

11.1.12.5. TB fat mass

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 5933 children in the Fracture Study, data on TB fat mass were available on all. Fat mass was not normally distributed (see Figure 42 below). The median fat mass was 7159g with the IQR 4787 to 10,925g. The mean ± SD for fat mass was 8500 ± 5102g. As the mean was approximately 20% different to the median and the SD was large, a logarithmic transformation was performed.

Figure 42: Distribution of fat mass for the children in the Fracture Study

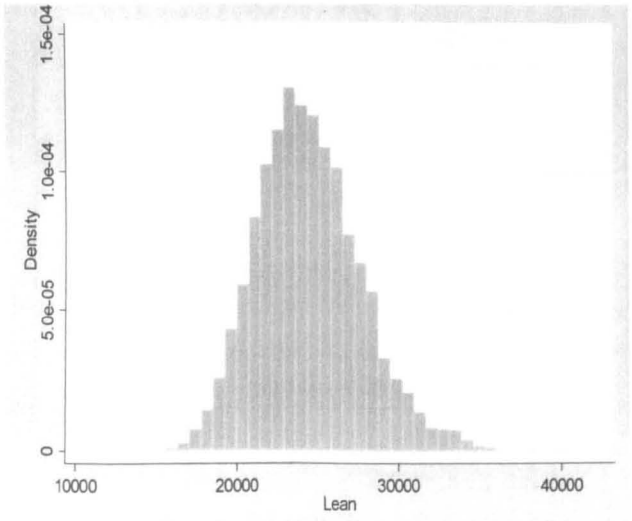


Median 7159g
IQR 4787 to 10,925g

11.1.12.6. TB lean mass

The method of data collection for this variable has been described in Methods, Chapter 8, page 189. Of the 5933 children in the Fracture Study, data on TB lean mass were available on all. Lean mass was approximately normally distributed (see Figure 43 below) with a mean ± SD of 24,501g ± 3216.

Figure 43: Distribution of lean mass for the children in the Fracture Study



Mean 24,501g
SD 3216g

11.1.12.7. Humeral measurements

The method of data collection for this variable has been described in Methods, Chapter 9, page 203. Of the 5933 children in the Fracture Study, data on humeral measurements were available on 1557 (26.2%). The distributions were similar to those on pages 205 to 208, except with the larger sample size humeral area and BMC were normally distributed.

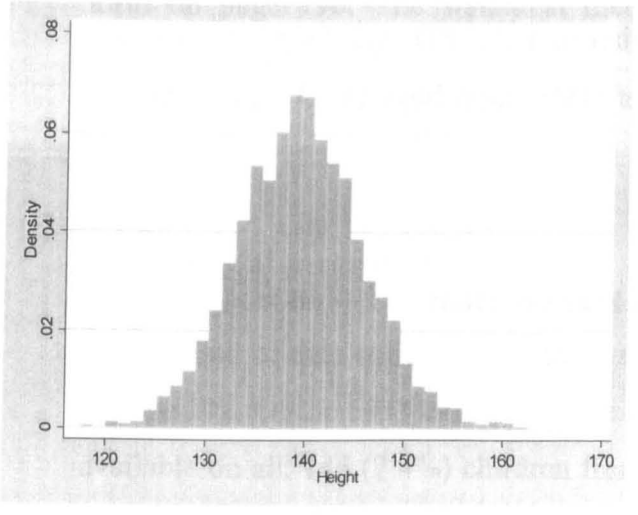
11.1.13. Anthropometry measured at same time as DXA scan

11.1.13.1. Height at time of DXA scan

The method of data collection for this variable has been described in Methods, Chapter 8, page 191. Of the 5933 children in the Fracture Study, data on height were available on all. Height was approximately normally distributed (see Figure 44 on the next page)

with a mean ± SD of 139.6cm ± 6.4cm. Boys were taller than girls (139.9cm versus 139.3cm P<0.001).

Figure 44: Distribution of height for the children in the Fracture Study

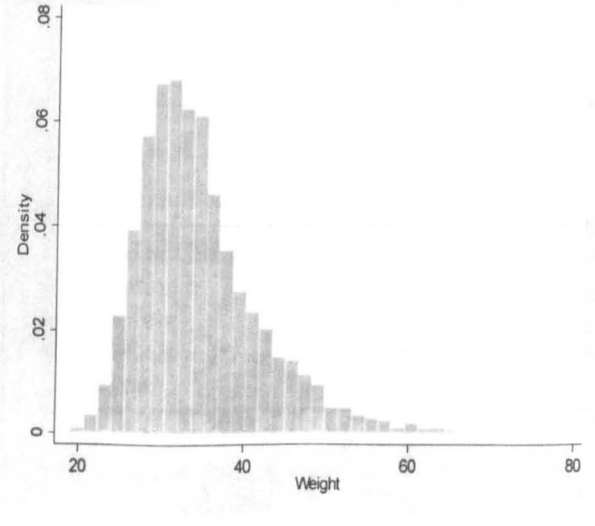


Mean 139.6cm
SD 6.4cm

11.1.13.2. Weight at time of DXA scan

The method of data collection for this variable has been described in Methods, Chapter 8, page 191. Of the 5933 children in the Fracture Study, data on weight were available on all. Weight was not normally distributed (see Figure 45 below). The median weight was 33.0kg with the IQR 29.4 to 38.2kg. The mean ± SD for weight was 34.6 ± 7.4kg. As the mean was more than 3% different to the median and the SD was large a 1/(square-root) transformation was performed. Girls were heavier than boys (34.9 kg versus 34.3 kg, P=0.003).

Figure 45: Distribution of weight for the children in the Fracture Study

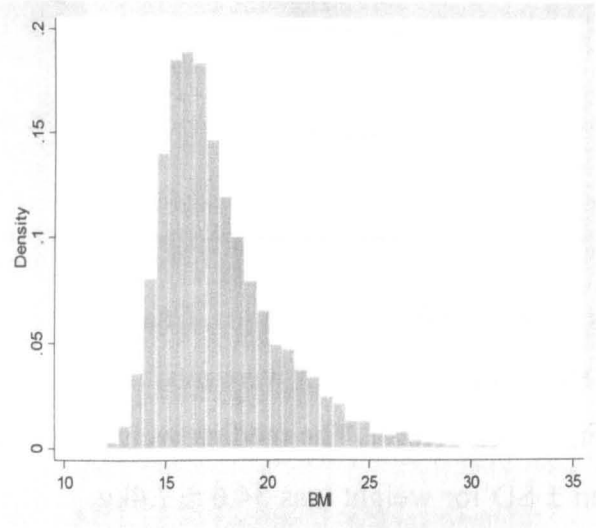


Median 33kg
IQR 29.4 to 38.2kg

11.1.13.3. BMI at time of DXA scan

The method of data collection for this variable has been described in Methods, Chapter 8, page 191. Of the 5933 children in the Fracture Study, data on BMI were available on all. BMI was positively skewed (see Figure 46 below). The median BMI was 17.0kg/m^2 with the IQR 15.6 to 19.0 kg/m^2. The mean ± SD for BMI was 17.7 ± 2.9 kg/m^2. As the mean was more than 3% different to the median and the SD was large an inverse transformation was performed. Girls had greater BMIs than boys (17.9 kg/m^2 versus 17.4 kg/m^2, P<0.001).

Figure 46: Distribution of BMI for children in the Fracture Study



Median 17.0 kg/m^2
IQR 15.6 to 19.0 kg/m^2

11.2. MATERNAL DATA

A summary of the maternal data used in the Fracture Study has been shown in tabular form on page 196. The statistical methods used to describe the maternal data are discussed on page 222.

11.2.1. Size of pregnancy

The method of data collection for this variable has been described in Methods, Chapter 8, page 192. Of the 5933 children in the Fracture Study, data on size of pregnancy were available on all. 145 (2.4%) children from the Fracture Study were non-singleton births i.e. twins or triplets. Size of pregnancy was included in all analyses to allow for potential clustering effects of being a twin or singleton, by using as a potential confounder. There were no gender differences in size of pregnancy.

11.2.2. Smoking in pregnancy

The method of data collection for this variable has been described in Methods, Chapter 8, page 192. Of the 5933 children in the Fracture Study, data on maternal smoking during pregnancy were available on 5543 (93.4%). Of these 5543, 678 (12.2%) had smoked during the second trimester of pregnancy. There was a strong social gradient of smoking during pregnancy (see Table 48 below), P<0.001.

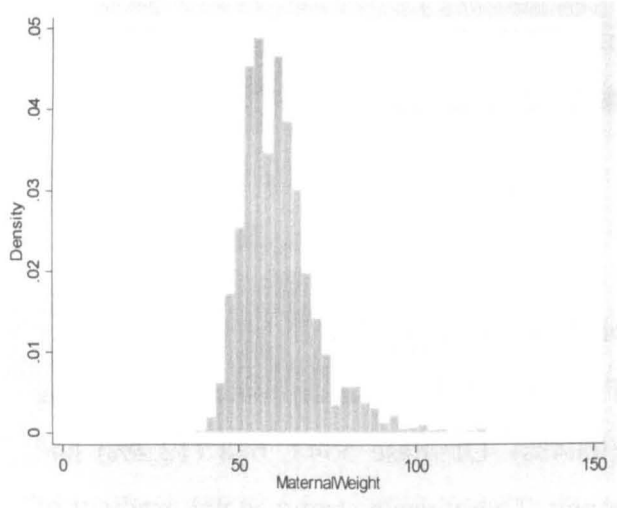
Table 48: Distribution of mothers who smoked during pregnancy according to educational level

Maternal education	Non smokers N=4778 N (%)	Smokers N=641 N (%)
none or CSEs	510 (10.7)	151 (23.6)
vocational	358 (7.5)	70 (10.9)
O levels	1655 (34.6)	262 (40.9)
A levels	1387 (29.0)	115 (17.9)
degree	868 (18.2)	43 (6.7)

11.2.3. Maternal pre-pregnancy weight

The method of data collection for this variable has been described in Methods, Chapter 8, page 192. Of the 5933 children in the Fracture Study, data on maternal weight were available on 5205 (87.7%). Maternal weight was positively skewed (see Figure 47 below). The median weight was 60.5kg with the IQR 54.1 to 66.8kg. The mean ± SD for maternal weight was 61.8 ± 10.5 kg/m^2. As the mean was less than 3% different to the median and the SD was not large a transformation was not considered necessary.

Figure 47: Distribution of maternal pre-pregnancy weight for children in the Fracture Study

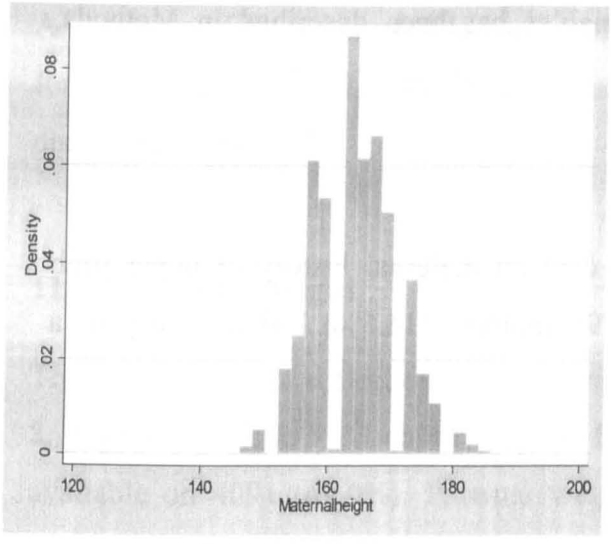


Median 60.5kg
IQR 54.1 to 66.8kg

11.2.4. Maternal height

The method of data collection for this variable has been described in Methods, Chapter 8, page 192. Of the 5933 children in the Fracture Study, data on maternal height were available on 5425 (91.4%). Maternal height was approximately normally distributed (see Figure 48 on next page) with a mean ± SD of 164.2cm ± 6.6cm.

Figure 48: Distribution of maternal height for the children in the Fracture Study

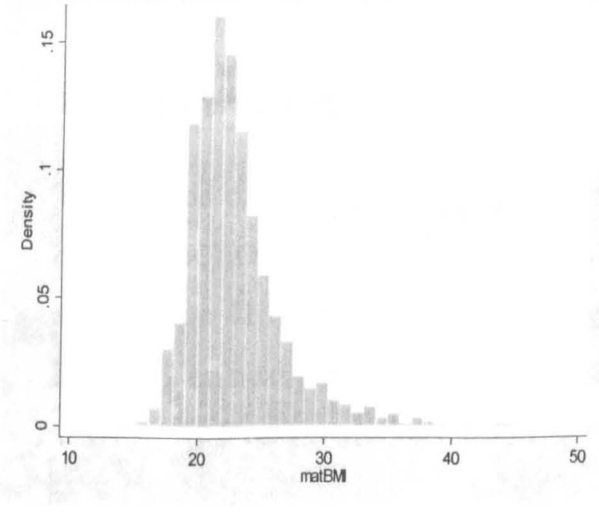


Mean 164.2cm
SD 6.6cm

11.2.5. Maternal pre-pregnancy BMI

The method of data collection for this variable has been described in Methods, Chapter 8, page 192. Of the 5933 children in the Fracture Study, data on maternal BMI were available on 5165 (87.1%). Maternal BMI was positively skewed (see Figure 49 below). The median BMI was 22.2 kg/m^2 with the IQR 20.5 to 24.3 kg/m^2. The mean ± SD for maternal BMI was 22.9 ± 3.7 kg/m^2. As the mean was more than 3% different to the median and the SD was large a logarithmic transformation was performed.

Figure 49: Distribution of maternal pre-pregnancy BMI for children in the Fracture Study



Median 22.2 kg/m^2
IQR 20.5 to 24.3 kg/m^2

11.2.6. History of fractures

The method of data collection for these variables has been described in Methods, Chapter 8, page 193.

11.2.6.1. History of upper limb fracture

Of the 5933 children in the Fracture Study, data on maternal history of upper limb fractures were available on 5449 (91.8%). 790 mothers (14.5%) had a history of a previous upper limb fracture.

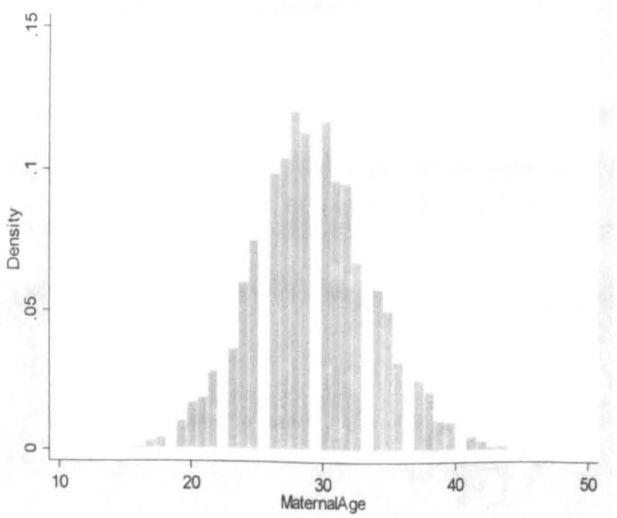
11.2.6.2. History of lower limb fracture

Of the 5933 children in the Fracture Study, data on maternal history of lower limb fractures were available on 5457 (92.0%). 363 mothers (6.7%) had a history of a previous lower limb fracture.

11.2.7. Age at delivery

The method of data collection for this variable has been described in Methods, Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on maternal age at delivery were available on 5645 (95.2%). Maternal age was approximately normally distributed (see Figure 50 below) with a mean ± SD of 29.2 years ± 4.5 years. There was a strong social gradient of maternal age with mums having no formal qualifications or CSEs being younger than mums with degrees (28.4 years versus 31.7 years, P<0.001).

Figure 50: Distribution of maternal age for the children in the Fracture Study



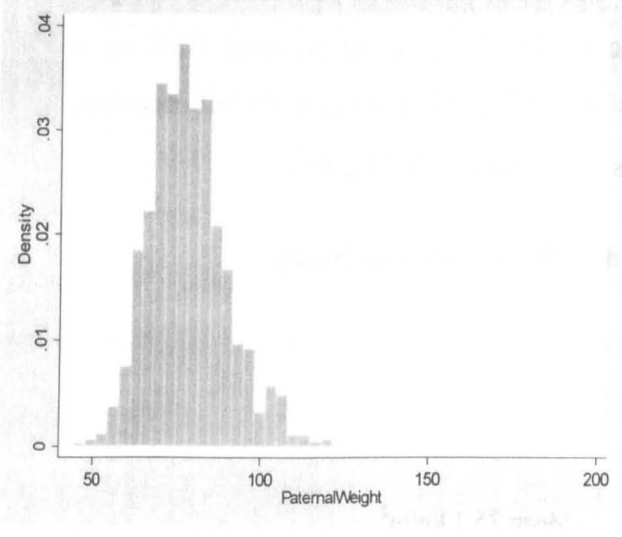
11.3. PATERNAL DATA

A summary of the paternal data used in the Fracture Study has been shown in tabular form on page 196. The statistical methods used to describe the paternal data are discussed on page 222.

11.3.1. Paternal weight

The method of data collection for this variable has been described in Methods, Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on paternal weight were available on 4091 (69.0%). Paternal weight was approximately normally distributed (see Figure 51 below) with a mean ± SD of 78.2kg ± 11.4kg.

Figure 51: Distribution of paternal weight for the children in the Fracture Study

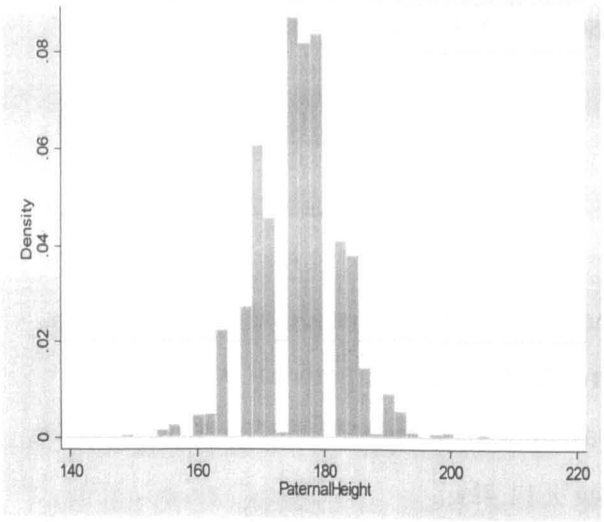


Mean 78.2 kg
SD 11.4 kg

11.3.2. Paternal height

The method of data collection for this variable has been described in Methods, Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on paternal height were available on 4101 (69.1%). Paternal height was approximately normally distributed (see Figure 52 on next page) with a mean ± SD of 176.4cm ± 6.8cm.

Figure 52: Distribution of paternal height for the children in the Fracture Study

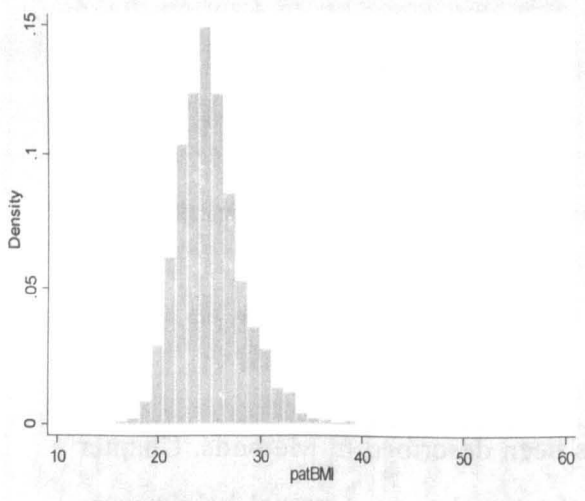


Mean 176.4cm
SD 6.8cm

11.3.3. Paternal BMI

The method of data collection for this variable has been described in Methods Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on paternal BMI were available on 4065 (68.5%). Paternal BMI was approximately normally distributed (see Figure 53 below). The mean ± SD for BMI was 25.1 kg/m^2 ± 3.3 kg/m^2.

Figure 53: Distribution of paternal BMI for children in the Fracture Study



Mean 25.1 kg/m^2
SD 3.3 kg/m^2

11.3.4. History of fractures

11.3.4.1. Upper limb fractures

The method of data collection for this variable has been described in Methods, Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on paternal history of upper limb fractures were available on 4076 (68.7%). Of these, 1028 (25.2%) of children had fathers who had a previous upper limb fracture.

11.3.4.2. Lower limb fractures

The method of data collection for this variable has been described in Methods, Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on paternal history of lower limb fractures were available on 4078 (68.7%). Of these, 639 (15.7%) of children had fathers who had a previous lower limb fracture.

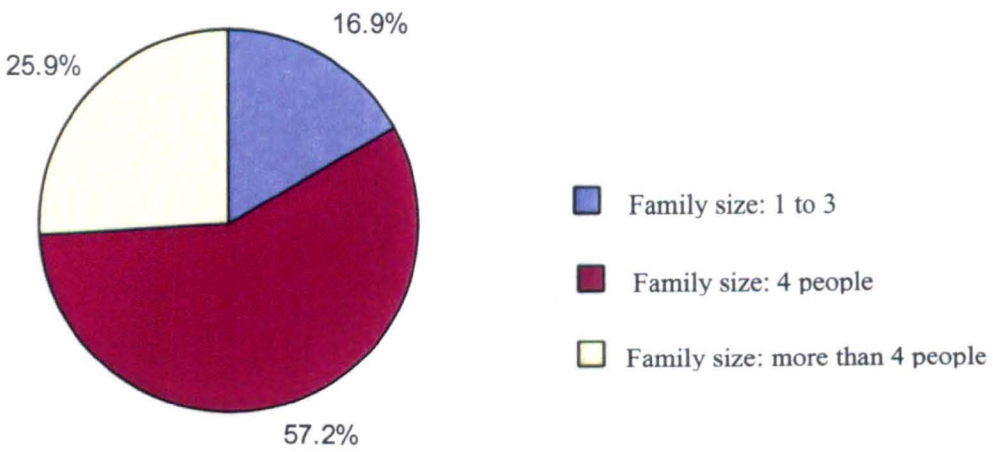
11.4. FAMILY DATA

A summary of the family data used in the Fracture Study has been shown in tabular form on page 196. The statistical methods used to describe the family data are discussed on page 222.

11.4.1. Family size

The method of data collection for this variable has been described in Methods, Chapter 8, page 193. Of the 5933 children in the Fracture Study, data on family size were available on 5060 (85.3%). See Figure 54 below for distribution.

Figure 54: Distribution of family size for the children in the Fracture Study



11.5. SOCIO-ECONOMIC DATA

A summary of the socio-economic data used in the Fracture Study has been shown in tabular form on page 196. 4346 (73.3%) of children in the Fracture Study had data on all measures of socio-economic status. The statistical methods used to describe the socio-economic data are discussed on page 222.

11.5.1. Housing tenure

The method of data collection for this variable has been described in Methods, Chapter 8, page 194. Of the 5933 children in the Fracture Study, data on housing tenure were available on 5350 (90.2%). Of these, 4634 (86.6%) lived in mortgaged or owned accommodation, 270 (5.1%) in privately rented accommodation and 446 (8.3%) in council or housing association rented accommodation.

11.5.2. Parental education

11.5.2.1. Maternal

The method of data collection for this variable has been described in Methods, Chapter 8, page 194. Of the 5933 children in the Fracture Study, data on maternal education were available on 5486 (92.5%). Of these, 16.7% of children in the Fracture Study had mothers educated to degree level (see Table 49 below).

Table 49: Distribution of maternal education for children in the Fracture Study

Maternal education	All N=5486 N (%)
none or CSEs	675 (12.3)
vocational	440 (8.0)
O levels	1936 (35.3)
A levels	1520 (27.7)
degree	915 (16.7)

11.5.2.2. Paternal

The method of data collection for this variable has been described in Methods, Chapter 8, page 194. Of the 5933 children in the Fracture Study, data on paternal education were available on 5353 (90.2%). Of these, 22.7% of children in the Fracture Study had fathers educated to degree level (see Table 50 below).

Table 50: Distribution of paternal education for children in the Fracture Study

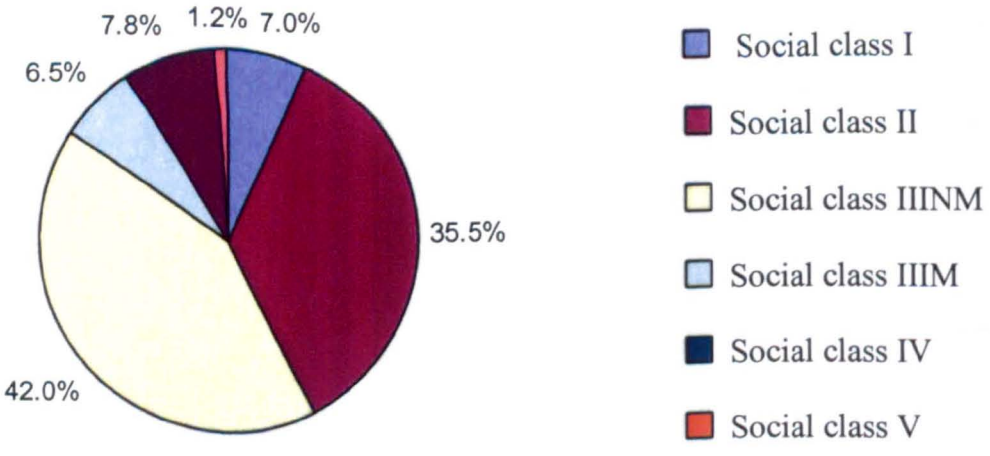
Paternal education	All N=5353 N (%)
none or CSEs	1010 (18.9)
vocational	428 (8.0)
O levels	1168 (21.8)
A levels	1533 (28.6)
degree	1214 (22.7)

11.5.3. Parental social class

11.5.3.1. Maternal

The method of data collection for this variable has been described in Methods, Chapter 8, page 195. Of the 5933 children in the Fracture Study, data on maternal social class were available on 4784 (80.6%). See Figure 55 below.

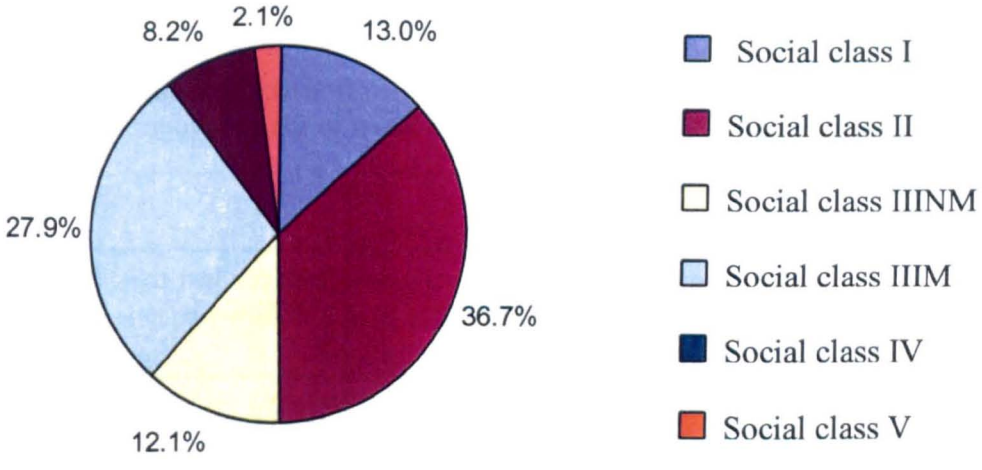
Figure 55: Distribution of maternal social class for children in the Fracture Study



11.5.3.2. Paternal

The method of data collection for this variable has been described in Methods, Chapter 8, page 195. Of the 5933 children in the Fracture Study, data on paternal social class were available on 5082 (85.7%). See Figure 56 below.

Figure 56: Distribution of paternal social class for children in the Fracture Study



11.6. COMPARISON OF FRACTURE STUDY CHILDREN WITH REST OF ALSPAC COHORT

Of the 5933 children in this study, 2901 (48.9%) were male. This shows a modest loss of male children. Other comparisons with the original ALSPAC cohort show that the Fracture Study population has less non-white children, less children from families living in council rented accommodation, less children with mothers who obtained no formal qualifications or CSEs only, and less children with fathers from social class V. (see Table 51 below).

Table 51: A comparison of children used in the Fracture Study with the whole ALSPAC cohort. P values are for the difference between those children in the Fracture Study and those in ALSPAC and are calculated by Chi-squared.

	Original ALSPAC cohort		Fracture Study		Children lost to follow-up		P value for difference
	N	%	N	%	N	%	
Gender							< 0.001
male	7538	51.5	2901	48.9	4367	53.3	
female	7092	48.5	3032	51.1	4060	46.7	
Ethnicity							< 0.001
white	11541	95.0	5219	96.5	6322	93.8	
non-white	611	5.0	191	3.5	420	6.2	
Housing tenure							< 0.001
mortgaged/owned	9880	76.0	4634	86.6	5246	68.6	
private rental	961	7.4	270	5.0	691	9.0	
council rental	2153	16.6	446	8.4	1707	22.3	
Maternal education							< 0.001
degree	1607	12.9	915	16.7	692	9.9	
A level	2809	22.5	1520	27.7	1289	18.4	
O level	4326	34.6	1936	35.3	2390	34.1	
vocational	1229	9.8	440	8.0	789	11.3	
CSE/none	2522	20.2	675	12.3	1847	26.4	
Paternal social class							< 0.001
I	1206	10.9	661	13.0	545	9.2	
II	3754	34.1	1865	36.7	1889	31.8	
III non manual	1199	10.9	615	12.1	584	9.8	
III manual	3465	31.4	1419	27.9	2046	34.5	
IV	1079	9.8	414	8.2	665	11.2	
V	317	2.9	108	2.1	209	3.5	

11.7. SUMMARY

The variables from ALSPAC used in the Fracture Study can be divided into continuous or categorical variables. The continuous variables are summarised in Table 52, below. Only gender, size of pregnancy, the DXA measures and the anthropometry measured at the same research clinic as the DXA scan had full data. All other variables had missing data. There has been preferential loss of those children from lower socio-economic backgrounds.

Table 52: Description of the continuous variables and any transformations required prior to being included in regression analyses

Variable name	*Distribution*	*Transformation to be used in regression analyses*
Child data		
gestational age	negatively skewed	not needed
birth weight	normal	
calcium intake	positively skewed	square-root
vitamin D intake	positively skewed	logarithmic
total energy intake	normal	
age at puberty measure	positively skewed	not needed
age at DXA scan	positively skewed	not needed
TBLH BMD	normal	
TBLH BMC	normal	
TBLH BA	normal	
fat mass	positively skewed	logarithmic
lean mass	normal	
humeral length	normal	
humeral width	normal	
humeral area	normal	
height	normal	
weight	positively skewed	1/(square-root)
BMI	positively skewed	inverse
Maternal data		
weight	positively skewed	not needed
height	normal	
BMI	positively skewed	logarithmic
age at delivery	normal	
Paternal data		
height	normal	
weight	normal	
BMI	normal	

Abbreviations: BA bone area; BMC bone mineral content; BMD bone mineral density; BMI body mass index; TBLH total body less head

CHAPTER 12: CONFOUNDING STRUCTURE OF BONE MASS IN CHILDREN AGED 9.8 YEARS

The previous results chapter (Chapter 11) described the distribution of the variables used in the Fracture Study. This chapter explores the confounding structure of bone mass: focussing on social determinants of bone mass and the association between body composition and bone mass. Three papers have been published in peer review journals based on this chapter (see Appendix I page 426, Appendix J page 434 and Appendix K page 442). The next chapter (Chapter 13) describes early life, maternal and paternal determinants of fractures, while Chapter 14 investigates the association between bone mass and fractures in children.

12.1. INTRODUCTION

Environmental stimuli during pregnancy or early life may affect skeletal growth in later life by programming (see Literature Review, Chapter 2, page 44). Social position or socio-economic status, used to define social inequality, is a factor that may affect bone mass via programming. It is usually measured by occupation, income or educational achievement (495). Education is the component of socio-economic status that consistently predicts health for women and their children (496). Low educational achievement, particularly in high-income countries, limits access to jobs and other social resources, which in turn limits capacity to integrate within society and increases the risk of subsequent poverty.

No previous studies have been published on the associations between deprivation and bone density measured in childhood (see Literature Review, Chapter 3, page 88), but intuitively, it can be felt that increasing deprivation is likely to be associated with lowered bone mass or density. In adults it is well recognised that there are socio-economic gradients in some of the risk factors for osteoporosis, specifically nutrition (300), BMI (497), smoking (498), alcohol consumption (501) and levels of physical activity (499). But the data for a socio-economic gradient for bone mass in adults is conflicting. Some authors have found no evidence for an association between socio-

economic factors and bone mass in adults (308,499,500). Others have found a positive association where increasing socio-economic status is associated with increased bone mass (302-306,501). One study found a negative association where increasing socio-economic status was associated with decreased bone mass (307). All of these studies used different measures of socio-economic status, and so are difficult to compare. Therefore, it is hypothesized that deprivation experienced during intra-uterine life may programme bone mass in childhood and thus may directly contribute to lowered peak bone mass.

Body composition, particularly body weight, is well recognised as being an important determinant of bone mass in adults (502). Body composition is also well recognised to influence the results of DXA scans, the most commonly used tool for measuring bone mass in children (see Literature Review, Chapter 2, page 48). Body composition can be assessed by measuring many variables, such as height, body weight, BMI, fat mass or lean mass. However, body weight or BMI are an ill-defined combination of fat mass, lean mass, bone mass and height. This potential confusion is highlighted by the contradictory literature on the relationship between obesity and bone mass in childhood (see Literature Review, Chapter 3, page 89). However, lean mass is a consistent positive predictor of bone mass (see Literature Review, Chapter 3, page 89), but the association between fat mass and childhood bone mass is contradictory (see Literature Review, Chapter 3, page 89). No studies were found that investigated the association between height and bone mass in childhood.

12.2. AIMS OF THIS CHAPTER

The aims of this chapter were to investigate the confounding structure of the main exposure variable for the Fracture Study. Two specific areas were focussed on: socio-economic status and body composition. It is likely that socio-economic position and body composition are themselves inter-related. Therefore the specific aims were

1. To investigate the association between socio-economic status and body composition in a contemporary cohort of children

2. To carry out the first investigation into the potential programming effects of social position measured during pregnancy on bone mass in children measured at aged 9.8 years.

3. To investigate the association between body composition and bone mass in children, specifically

(a) To confirm the association between lean mass and bone mass

(b) To investigate the association between height and bone mass

(c) To investigate the association between fat mass and bone mass, particularly bone area, and whether this is independent of height and lean mass

(d) To investigate whether fat mass preferentially influences bone growth at weight bearing sites

(e) To investigate whether fat mass distribution (i.e. trunkal or peripheral) influences bone mass

12.3. METHODS

12.3.1. Study population

The study population is described by Figure 28, Methods, Chapter 9, page 214 and consists of 5933 children from ALSPAC, a birth cohort study (see Methods, Chapter 8, page 179). The inclusion criteria for the Fracture Study were that the children had attended a research clinic at aged 9.8 years for a DXA scan, and further research clinics at aged 10.7 years and also at aged 11.7 years. Ethical approval for this study had obtained from the ALSPAC Law and Ethics Committee and from three LRECs (see Methods, Chapter 9, page 202).

12.3.2. Measures of social position

The measures of social position used in this chapter were housing tenure, parental education and parental social class. The methods of data collection have been described in Methods, Chapter 8, page 194. The basic distribution of the measures of social position are described in Results, Chapter 11, page 253. For the purposes of this chapter maternal education has been coded 1 for no formal qualifications or CSEs, 2 or vocational qualifications, 3 for O levels, 4 for A levels and 5 for degree.

12.3.3. Measures of body composition at aged 9.8 years

For this chapter the measures of body composition used were height and weight at aged 9.8 years and fat mass and lean mass obtained from the DXA scans performed at aged 9.8 years. The methods of data collection have been described in Methods, Chapter 8, page 191.

12.3.4. Measures of bone mass at aged 9.8 years

12.3.4.1. Measures of bone size

TBLH bone area was measured using a Lunar Prodigy DXA scanner at aged 9.8 years. The method of data collection have been described in Methods, Chapter 8, page 189.

Regional analysis of the spine, upper and lower limbs was also undertaken (see Methods, Chapter 8, page 189). A sub-sample of children had regional analyses of the humerus performed (see Methods, Chapter 9, page 203). The following measures of bone size were used in this chapter: total body less head (TBLH) bone area, humerus length, humerus width, humerus area, and humeral aspect ratio. Humeral aspect ratio (AR) was used because it may give additional information on fracture risk, as according to beam theory, columns with smaller aspect ratios have a reduced fracture risk compared to columns with larger aspect ratios. It was calculated as humeral length divided by width.

12.3.4.2. Estimates of volumetric bone density

TBLH BMC was measured using a Lunar Prodigy DXA scanner at aged 9.8 years. The method of data collection have been described in Methods, Chapter 8, page 189. TBLH BMC adjusted for body size (height, weight, and TBLH bone area), was used to estimate total body volumetric density. From the regional humeral analyses, estimated volumetric density (humeral vBMD) was calculated by assuming the humerus was a cylinder and dividing humeral BMC by humeral volume derived by the following equation:

Humeral volume = humeral length X humeral cross-sectional area (Πr^2)

where r was calculated as half the humeral width.

12.3.5. Other measures

Age at the time of the DXA scan was recorded (see Methods, Chapter 8, page 189). Data on gender (see Methods, Chapter 8, page 184), ethnicity (see Methods, Chapter 8, page 184), size of pregnancy (see Methods, Chapter 8, page 192) and pubertal status (see Methods, Chapter 8, page 189) had been previously collected.

12.3.6. Statistical analyses

All statistical analyses were performed with Stata 8.0 (see Methods, Chapter 10, page 219). A two-tailed unpaired t test was used to assess differences between binary variables such as gender.

12.3.6.1. Analysing the relationship between social position and body composition

To analyse the association between social position and body composition, means ± 95%CI of body composition were calculated for the individual categories of each measure of social position using linear regression. These were initially run adjusted for age at measurement of body composition, and then progressively adjusted for gender, ethnicity and size of pregnancy using multivariable linear regression. Analyses were additionally adjusted for size of pregnancy to allow for potential clustering effects of being a twin (see Methods, Chapter 11, page 245). Analyses were further adjusted for pubertal stage. All analyses were further adjusted for height and height-squared to take account of differences in stature.

P values were the test for trend, calculated by treating the measures of social position as continuous variables in the regression models. Participants with missing data were excluded from analysis. Stratified analyses were then carried out to look for evidence of effect modification or interaction (see Methods, Chapter 10, page 226). Interactions between variables were assessed by including a multiplicative interaction term in the regression models and calculating the likelihood ratio test (LRT).

12.3.6.2. Analysing the association between social position and bone mass

To analyse the association between social position and bone mass, linear regression was used to calculate means ± 95%CI for the effects of each measure of social position on the measures of bone mass. These were initially run adjusted for age at measurement of bone mass, and then progressively adjusted for gender, ethnicity and size of pregnancy using multivariable linear regression. Analyses were further adjusted for pubertal stage. Participants with missing data were excluded from analysis. The regressions between social position and bone mass were then further adjusted for height, weight or height and weight using multivariable linear regression. In these analyses weight was transformed by a 1/(square-root) transformation (see Results, Chapter 11, page 243).

Lean mass was substituted for height and fat mass (log-transformed, see Results, Chapter 11, page 241) was substituted for weight. Stratified analyses were then carried out to look for evidence of effect modification or interaction (see Methods, Chapter 10, page 226). Interactions between variables were assessed by including a multiplicative interaction term in the regression models and calculating the likelihood ratio test (LRT). Finally, the associations between social position and bone size and between social position and estimated volumetric density were analysed separately by multivariable linear regression, adjusting for age at time of bone mass measurements, gender, ethnicity and size of pregnancy.

12.3.6.3. Analysing the relationship between body composition and bone mass

To analyse the association between body composition and bone size, linear regression was used to calculate regression coefficients ± 95%CI for the effects of each measure of body composition on the measures of bone mass. To allow comparisons, standardised variables were created by subtracting from the mean and dividing by the standard deviation. These were initially run adjusted for age at measurement of body composition and bone mass, and then progressively adjusted for gender, ethnicity, size of pregnancy and socio-economic status using multivariable linear regression. Analyses were further adjusted for pubertal stage. Participants with missing data were excluded from analysis.

To analyse the association between body composition and estimated volumetric density only TBLH BMD and humeral vBMD were used. This is because the best estimate of total body volumetric density (total body BMC adjusted for body composition) cannot be used to assess the effects of body composition on volumetric bone density. Multivariable linear regression was used to assess the effects of height, lean mass and fat mass on estimated volumetric bone density, adjusted for age, gender, ethnicity, size of pregnancy and socio-economic status. Weight was not used as it is an ill-defined composite of height, lean and fat mass (see introduction). Regressions were further adjusted for pubertal stage.

The regression between fat mass and bone mass was additionally adjusted for height, height and lean mass or height and height-squared to assess whether the association between fat mass and bone mass is independent of height and lean mass. To allow

comparisons, standardised variables were created by subtracting from the mean and dividing by the standard deviation. To investigate whether fat mass preferentially influences bone growth at weight-bearing sites, the relationship between total body fat mass and site-specific bone mass (trunk, upper limbs, lower limbs) was investigated by linear regression adjusted for age, gender, ethnicity, size of pregnancy, pubertal status, height and lean mass. To allow comparisons, standardised variables were created by subtracting from the mean and dividing by the standard deviation. To assess whether distribution of fat mass influenced bone growth, the association between trunkal fat or peripheral fat and TBLH bone mass was investigated by linear regression adjusted for age, gender, ethnicity, size of pregnancy, pubertal status, height and lean mass. To allow comparisons, standardised variables were created by subtracting from the mean and dividing by the standard deviation.

Stratified analyses were then carried out to look for evidence of effect modification or interaction (see Methods, Chapter 10, page 226). Interactions between variables were assessed by including a multiplicative interaction term in the regression models and calculating the likelihood ratio test (LRT).

12.4. RESULTS

12.4.1. Basic description of the study population

Of the 5993 children in this Fracture Study, 2901 (48.9%) were male and 191 (3.5%) were non-white (see Results Chapter 11, page 227). Comparison with the rest of the ALSPAC cohort shows preferential loss of male children and those of non-white ethnicity (see Results Chapter 11, page 256).

Data on measures of social position were missing for some children (see Results Chapter 11, page 253). The distributions of the measures of social position for the children in the Fracture Study have been described in Results Chapter 11, starting on page 253. Total number (%) of children in each category for all measures of social position for the whole Fracture Study cohort, and separately for boys and girls is shown in Table 53, on the next page. There were no gender differences in measures of social position.

Data on body composition and bone mass were available for all children. Distributions of height, weight, fat and lean mass, TBLH BMC, bone area and BMD for the children in the Fracture Study have been described in Results, Chapter 11, starting on page 240. Means ± SDs for body composition and bone mass in boys and girls is shown in Table 53, page 268. Boys were taller but lighter than girls. Boys had more lean mass but less fat mass than girls. Boys also had a greater BMC, a bigger bone area and greater BMD than girls. From the humerus measures girls had longer bones but boys had wider bones. Girls also had a greater humeral AR. There was no gender difference in humeral area or estimated volumetric density.

Table 53: Measures of social position and body composition in boys and girls in the Fracture Study

	Fracture Study children combined		Boys		Girls		P value for difference
	N	%	N	%	N	%	
Housing							0.287
mortgaged/owned	4634	86.6	2295	86.9	2339	86.3	
private rental	270	5.1	141	5.3	129	4.8	
council rental	446	8.3	205	7.8	241	8.9	
Maternal education							0.484
none/CSEs	675	12.3	329	12.2	346	12.4	
vocational	440	8.0	227	8.4	213	7.6	
O levels	1936	35.3	954	35.4	982	35.2	
A levels	1520	27.7	748	27.8	772	27.7	
degree	915	16.7	436	16.2	479	17.2	
Paternal education							0.105
none/CSEs	1010	18.9	472	18.0	538	19.7	
vocational	428	8.0	201	7.7	227	8.3	
O levels	1168	21.8	602	22.9	566	20.8	
A levels	1533	28.6	732	27.9	801	29.4	
degree	1214	22.7	619	23.6	595	21.8	
Maternal social class							0.439
I	336	7.0	163	7.0	173	7.1	
II	1697	35.5	827	35.3	870	42.8	
IInm	2007	42.0	1005	42.9	1002	41.1	
IIm	313	6.5	157	6.7	156	6.4	
IV	372	7.8	167	7.1	205	8.4	
V	59	1.2	26	1.1	33	1.4	
Paternal social class							0.123
I	661	13.0	330	13.4	331	12.7	
II	1865	36.7	921	37.3	944	36.1	
IInm	615	12.1	295	12.0	320	12.2	
IIm	1419	27.9	683	27.7	736	28.2	
IV	414	8.2	186	7.5	228	8.7	
V	108	2.1	52	2.1	56	2.1	

	Fracture Study children combined		Boys		Girls		
	Mean	SD	Mean	SD	Mean	SD	
Height (cm)	139.6	6.4	139.9	6.1	139.3	6.6	< 0.001
Weight (kg)	34.6	7.4	34.3	7.1	34.9	7.7	0.003
Fat mass (kg)	8.5	5.1	7.3	4.8	9.6	5.1	< 0.001
Lean mass (kg)	24.5	3.2	25.5	3.0	23.6	3.2	< 0.001
TBLH BMC (g)	890	183	904	175	877	190	< 0.001
TBLH bone area (cm^2)	1136	164	1147	155	1126	172	< 0.001
TBLH BMD (g/cm^2)	0.777	0.054	0.782	0.053	0.772	0.055	< 0.001
Humeral length (cm)	24.9	1.5	24.8	1.4	25.1	1.6	< 0.001
Humeral width (cm)	1.91	0.17	1.93	0.17	1.88	0.17	< 0.001
Humeral area (cm^2)	47.6	6.0	47.8	5.9	47.3	6.1	0.074
Humeral AR	13.15	1.09	12.93	1.09	13.38	1.05	< 0.001
Humeral vBMD (g/cm^3)	0.491	0.049	0.489	0.051	0.493	0.048	0.113

Abbreviations: AR aspect ratio; BMC bone mineral content; BMD bone mineral density; SD standard deviation; TBLH total body less head; vBMD volumetric bone density

12.4.2. Association between social position and body composition

12.4.2.1. Height

An association was seen between all measures of social position and height adjusted for age at height measurement. Children living in council rented accommodation were shorter than those living in mortgaged or owned accommodation (P<0.001). Similarly, children with parents with no formal qualifications or to CSEs only, were shorter than children whose parents had degrees (P<0.001 for both maternal and paternal education). Children with parents from lower social classes were shorter compared to children with parents from higher social classes (P<0.001 for maternal social class, and P=0.036 for paternal social class). Further adjustment for gender, ethnicity, size of pregnancy and pubertal stage did not change the associations. Using maternal education, after adjustment for age, gender, ethnicity and size of pregnancy there was a 0.9cm difference in height between the highest and lowest categories (see Figure 57 A, page 271). This difference was similar for housing tenure and paternal education and paternal social class. For maternal social class there was a 1.8cm difference in height between children whose mothers were in social class I and those whose mothers were in social class V.

12.4.2.2. Weight

An association was seen between parental education and weight adjusted for age at weight measurement. Children with parents with no formal qualifications or to CSEs only were heavier than children whose parents had degrees (P=0.013 for maternal education, and P=0.017 for paternal education). Further adjustment for gender, ethnicity and size of pregnancy did not change these associations. However, adjustment for height and height-squared, to allow for differences in stature, resulted in a strong negative association (P<0.001) between all measures of social position and weight i.e. children from lower social positions had a greater weight than children from higher social positions (see Figure 57 B, page 271). Additional adjustment for puberty did not change these associations. Using maternal education, after adjustment for age, gender, ethnicity, size of pregnancy and height there was a 1.1kg difference in weight between the highest and lowest categories.

12.4.2.3. Lean mass

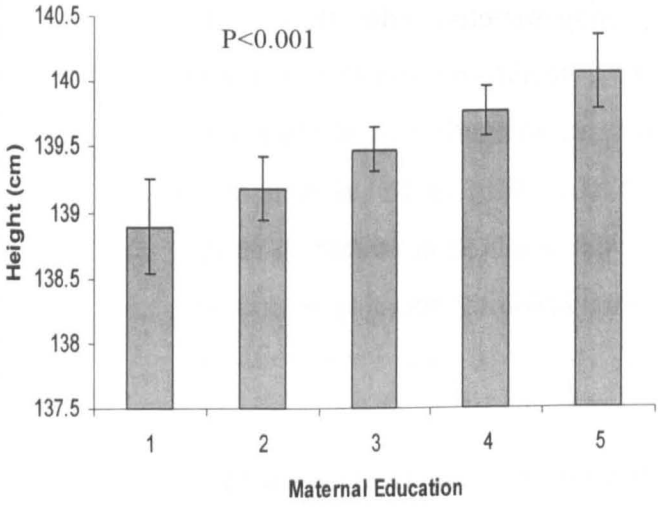
An association was seen between all measures of social position and lean mass adjusted for age at lean mass measurement. Children from higher social positions had a higher lean mass. Further adjustment for gender, ethnicity, size of pregnancy and pubertal stage did not change the associations. However, adjustment for height and height-squared, to allow for differences in stature, resulted in no association between any measure of social position and lean mass (see Figure 57 C, page 271). Stratification by height showed no evidence of effect modification or interaction and this was confirmed with the LRT test.

12.4.2.4. Fat mass

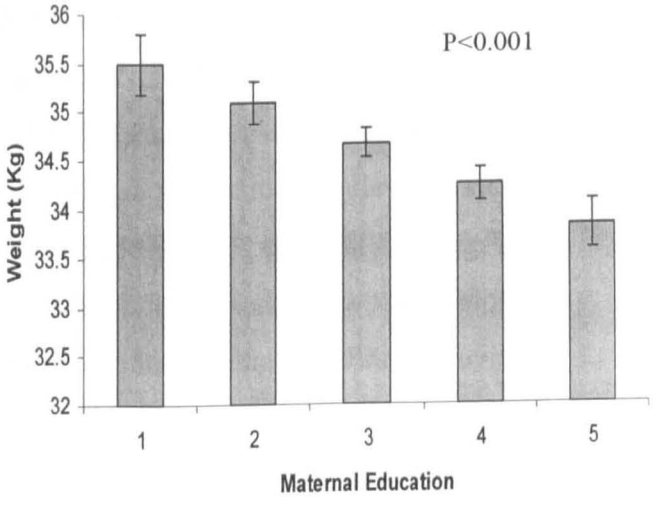
An association was seen between all measures of social position and fat mass adjusted for age at fat mass measurement except maternal social class. Children from lower socio-economic groups had a higher fat mass. Further adjustment for gender, ethnicity and size of pregnancy did not change the associations. However, adjustment for height and height-squared, to allow for differences in stature, resulted in a strong negative association ($P<0.001$) between all measures of social position and fat mass i.e. children from lower social positions had a greater fat mass than children from higher social positions (see Figure 57 D, page 271). Additional adjustment for puberty did not change these associations. For maternal education there was a 1.3 Kg difference in fat mass between the highest and lowest categories (see Figure 57 D, page 271). This difference was similar for all other measures of social position.

Figure 57: Bar charts showing associations between maternal education measured during pregnancy and body composition of offspring at aged 9.8 years.

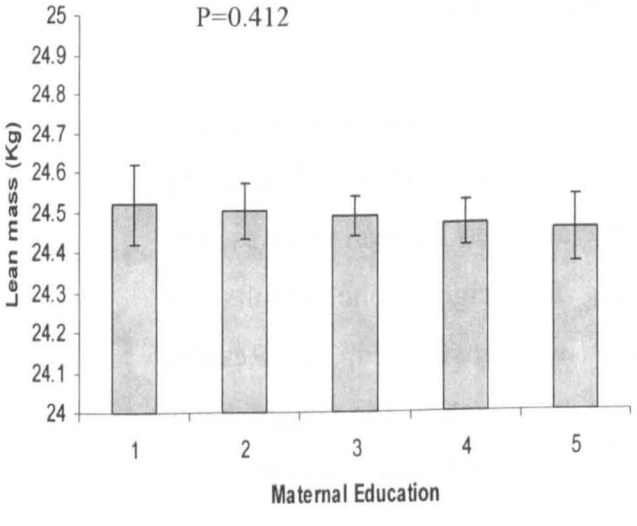
(A) Association between maternal education and height of offspring aged 9.8 years



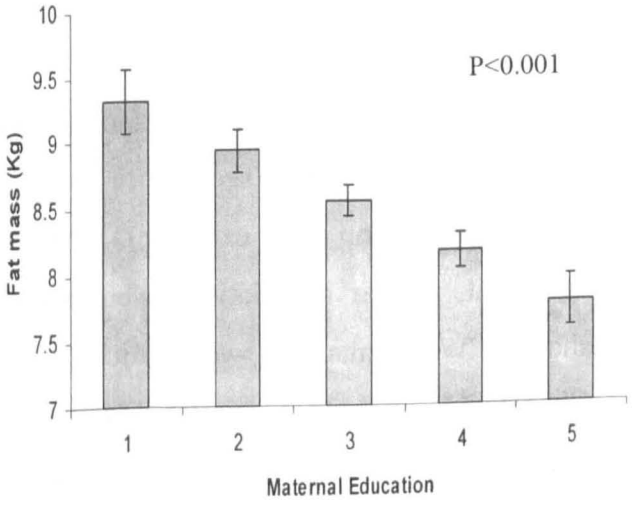
(B) Association between maternal education and weight of offspring aged 9.8 years



(C) Association between maternal education and lean mass of offspring aged 9.8 years



(D) Association between maternal education and fat mass of offspring aged 9.8 years



Graphs show mean ± 95%CI for measures of body composition in each category of maternal education (1=none/CSE, 2=vocational, 3=O level, 4=A level, 5=degree). Analyses are adjusted for age at time of body composition measurement, gender, ethnicity and size of pregnancy. Graphs (B), (C) and (D) are additionally adjusted for height and height-squared to allow for differences in stature. P values are test for trend.

12.4.3. Association between social position and bone mass

For this section the outcome of TBLH BMC and TBLH bone area were used. No association was seen between any measure of social position and either TBLH BMC or TBLH bone area after adjusting for age, gender, ethnicity or size of pregnancy (see Figure 58 A, page 274, and Figure 59 A page 275, using maternal education as an example). As shown in the previous section (page 269), social position is positively associated with height. Therefore, after adjusting for height, a negative association was seen between all measures of social position and either TBLH BMC or TBLH bone area (see Figure 58 B, page 274, and Figure 59 B page 275, using maternal education as an example) i.e. when height differences are taken into account, social position is negatively related to bone size and bone mineral content.

As also shown in the previous section (page 269) social position is negatively associated with weight. Literature review suggests that weight is positively associated with bone mass. Therefore, analyses were adjusted for weight (1/square-root transformed, see Results, Chapter 11, page 243), and a positive association was seen between all measures of social position except paternal social class and either TBLH BMC or TBLH bone area (see Figure 58 C, page 274, and Figure 59 C page 275, using maternal education as an example) i.e. when weight differences are taken into account, social position is positively related to bone size and bone mineral content.

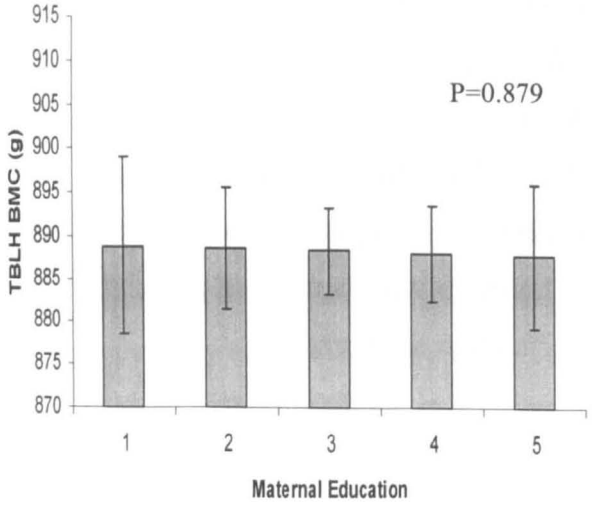
After adjusting for both height and weight, no association was seen between social position and bone mass (see Figure 58 D, page 274, and Figure 59 D page 275, using maternal education as an example). When analyses were adjusted for lean mass as well as height, similar associations were seen to adjusting for height alone (results not shown). When analyses were adjusted for fat mass (log transformed, see Results, Chapter 11, page 241), similar associations were seen to adjusting for weight alone (results not shown). Additional adjustment for puberty did not change these associations.

Finally, no association was seen between any measure of social position and any measure of bone size (TBLH bone area, humeral length, width, area or AR) after

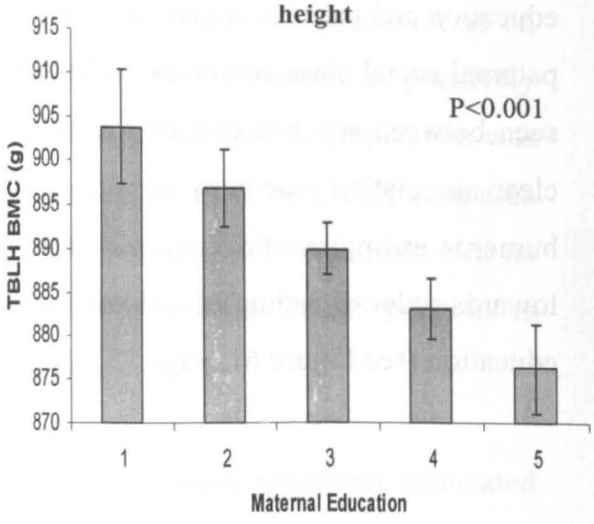
adjustment for age, gender, ethnicity and size of pregnancy (results not shown). After adjustment for height, a negative association was seen between social position and the total body measure of bone size as described above, but no consistent association was seen with any of the humeral measures of bone size (results not shown). After adjustment for weight, a positive association was seen between social position and the total body measure of bone size as described above, and also between parental education and paternal social class and humerus area, and between paternal education or paternal social class and humerus length (see Figure 60, page 276). No association was seen between any measure of social position and humerus width or humerus AR. No clear association was seen between any measure of social position and total body or humerus estimates of volumetric bone density, but there was a suggestion of a trend towards reduced estimated volumetric density of the humerus with increasing parental education (see Figure 61, page 277 using maternal education as an example).

Figure 58: Associations between social position and TBLH BMC, using maternal education as an example

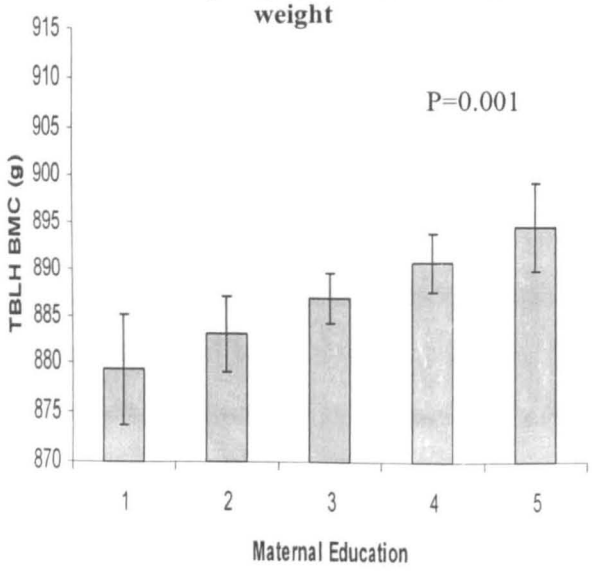
(A) Association between maternal education and TBLH BMC of offspring aged 9.8 years



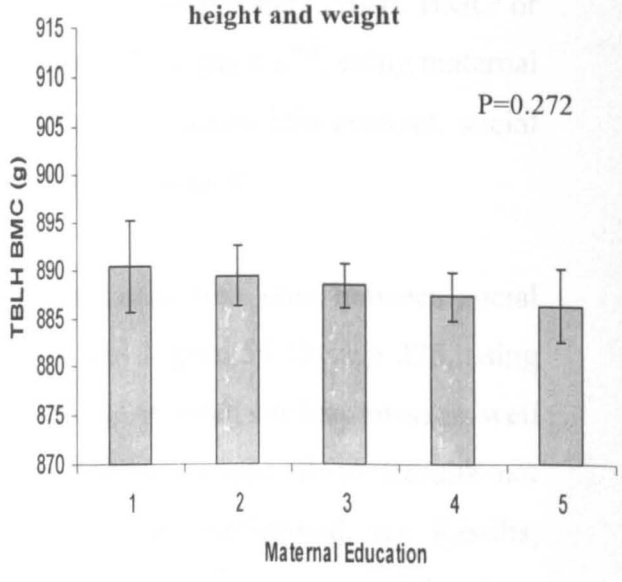
(B) Association between maternal education and TBLH BMC of offspring aged 9.8 years, adjusted for height



(C) Association between maternal education and TBLH BMC of offspring aged 9.8 years, adjusted for weight



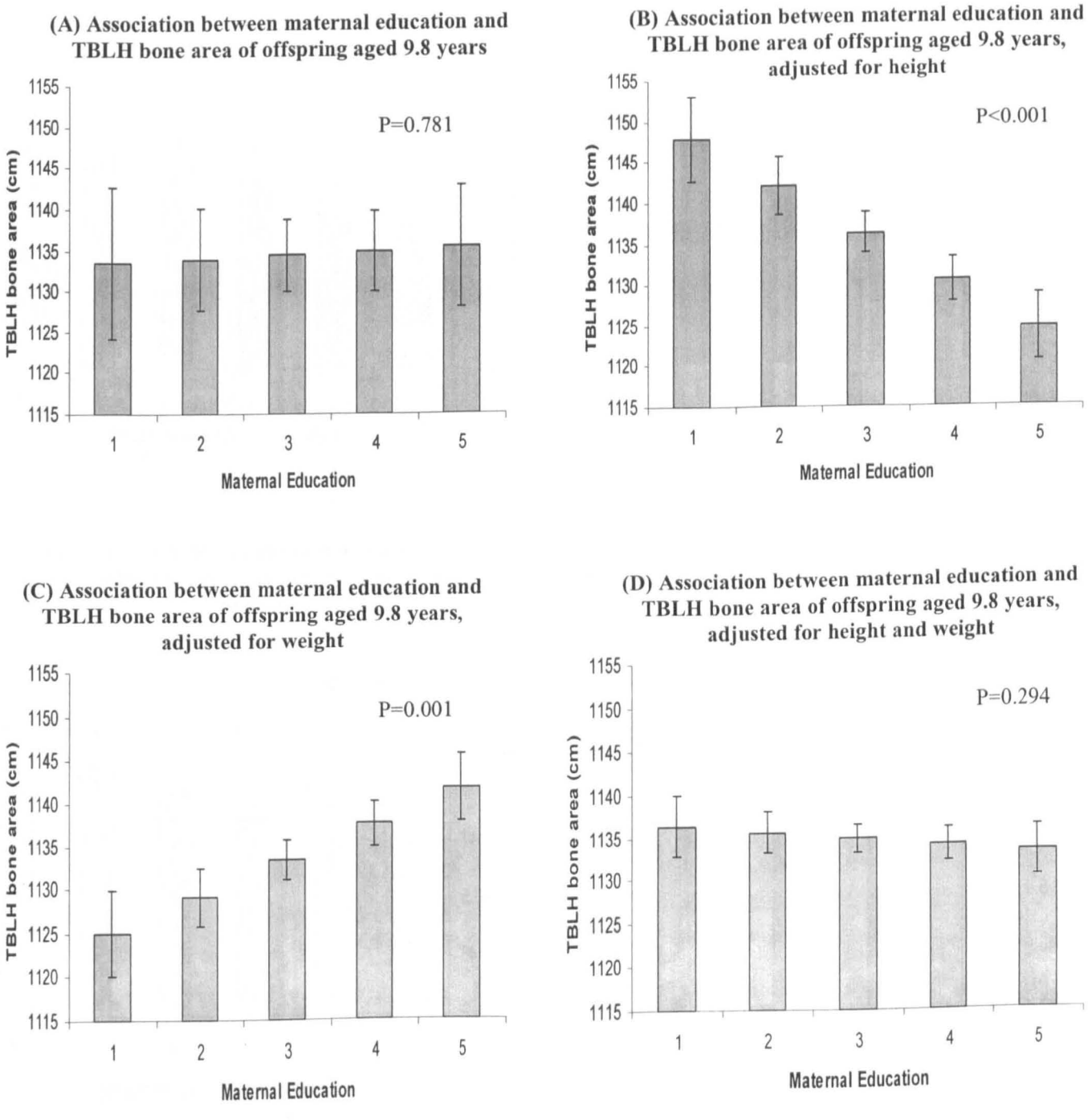
(D) Association between maternal education and TBLH BMC of offspring aged 9.8 years, adjusted for height and weight



Graphs show mean ± 95%CI for measures of TBLH BMC in each category of maternal education (1=none/CSE, 2=vocational, 3=O level, 4=A level, 5=degree). Analyses are adjusted for age at time of BMC measurement, gender, ethnicity and size of pregnancy. Graph (B) is additionally adjusted for height; graph (C) is additionally adjusted for weight; and graph (D) is additionally adjusted for height and weight. P values are test for trend.

Abbreviations: BMC bone mineral content; BMD bone mineral density; g grams; TBLH total body less head

Figure 59: Associations between social position and TBLH bone area, using maternal education as an example

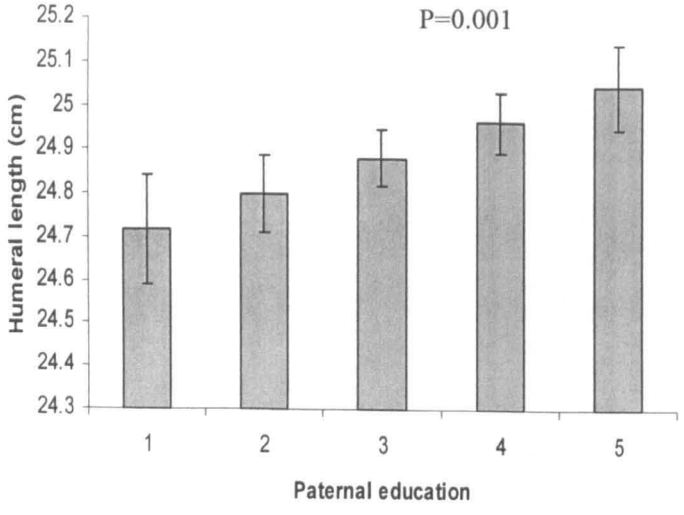


Graphs show mean ± 95%CI for measures of TBLH bone area in each category of maternal education (1=none/CSE, 2=vocational, 3=O level, 4=A level, 5=degree). Analyses are adjusted for age at time of BMC measurement, gender, ethnicity and size of pregnancy. Graph (B) is additionally adjusted for height; graph (C) is additionally adjusted for weight; and graph (D) is additionally adjusted for height and weight. P values are test for trend.

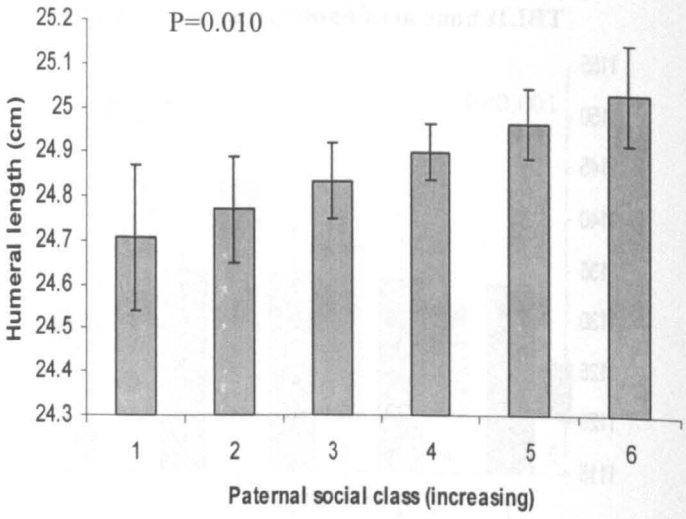
Abbreviations: cm centimetre; TBLH total body less head

Figure 60: Association between social position (paternal education or paternal social class) and humeral bone size, adjusted for body weight

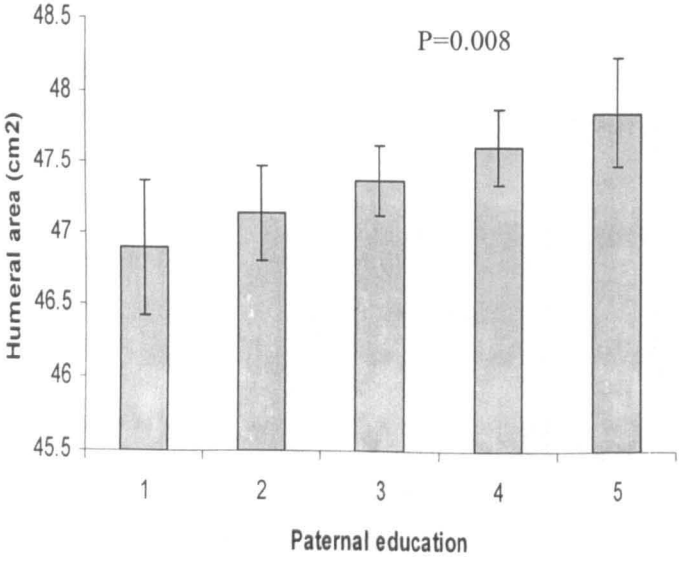
(A) Association between paternal education and humeral length of offspring aged 9.8, adjusted for weight



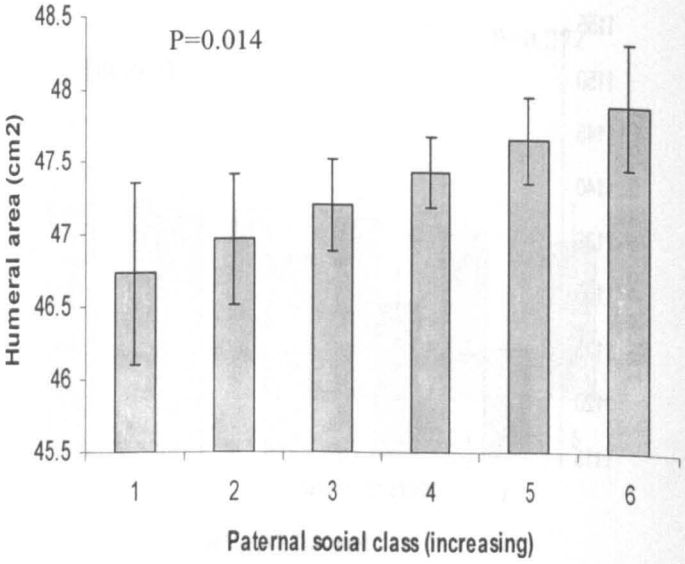
(B) Association between paternal social class and humeral length of offspring aged 9.8, adjusted for weight



(C) Association between paternal education and humeral area of offspring aged 9.8, adjusted for weight



(D) Association between paternal social class and humeral area of offspring aged 9.8, adjusted for weight

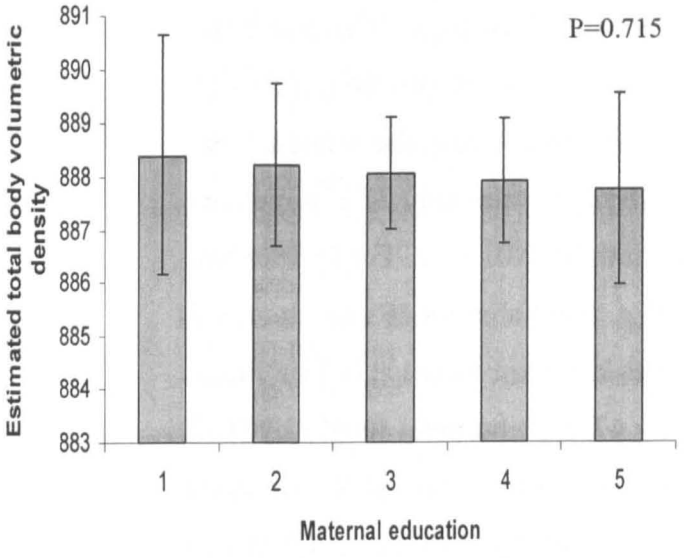


Graphs show mean ± 95%CI for measures of humerus bone size in each category of paternal education (1=none/CSE, 2=vocational, 3=O level, 4=A level, 5=degree) or paternal social class (1=V, 2=IV, 3=IIIm, 4=IIInm, 5=II, 6=I). Analyses are adjusted for age at time of bone size measurement, gender, ethnicity, size of pregnancy and weight. P values are test for trend.

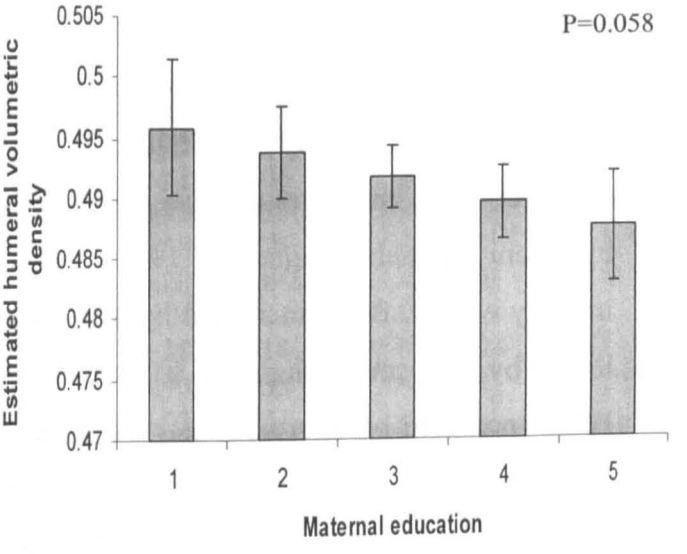
Abbreviations: cm centimetre

Figure 61: Association between social position, using maternal education as an example, and estimated volumetric bone density

(A) Association between maternal education and estimated total body volumetric bone density of offspring aged 9.8 years



(B) Association between maternal education and estimated humeral volumetric bone density of offspring aged 9.8 years



Graphs show mean ± 95%CI for measures of estimated bone density in each category of maternal education (1=none/CSE, 2=vocational, 3=O level, 4=A level, 5=degree). Analyses are adjusted for age at time of bone density measurement, gender, ethnicity and size of pregnancy. P values are test for trend.

12.4.4. Association between body composition and bone mass

12.4.4.1. Body composition and bone size

All measures of body composition (height, weight, lean mass and fat mass) were positively associated with measures of bone size (TBLH BMC, bone area and humeral length, width and area) adjusted for age. Weight and lean mass were negatively associated with humeral AR. Further adjustment for gender, ethnicity, size of pregnancy and pubertal stage did not change the associations. Because of the associations found between socio-economic status and both body composition (see page 269) and bone mass (see page 272) analyses were also further adjusted for social position, although results were unaffected. See Table 54, page 279. Without using the standardised variables, per 1 cm increase in height, BMC increased by approximately 22 g, bone area by 21 cm^2, humeral length by 0.19 cm and humeral width by 0.015 cm. Per kg increase in body weight, BMC increased by approximately 20 g, bone area by 19 cm^2, humeral length by 0.11 cm and humeral width by 0.012 cm. Per kilogram increase in lean mass, BMC increased by approximately 51 g, bone area by 47 cm^2, humeral length by 0.32 cm and humeral width by 0.039 cm. Per kilogram increase in fat mass, BMC increased by approximately 24g, bone area by 22 cm^2, humeral length by 0.12 cm and humeral width by 0.011 cm.

12.4.4.2. Body composition and BMD

Height, lean mass and fat mass were positively associated with total body BMD, (see Figure 62, left hand column, page 280), after adjustment for age, gender, ethnicity, size of pregnancy and socio-economic status. Further adjustment for pubertal stage did not change the results.

12.4.4.3. Body composition and estimated volumetric density

Fat mass was positively associated with humeral vBMD (see Figure 62, right hand column, page 280), after adjustment for age, gender, ethnicity, size of pregnancy and socio-economic status. However, height and lean mass were negatively associated with humeral vBMD. Further adjustment for pubertal stage did not change the results.

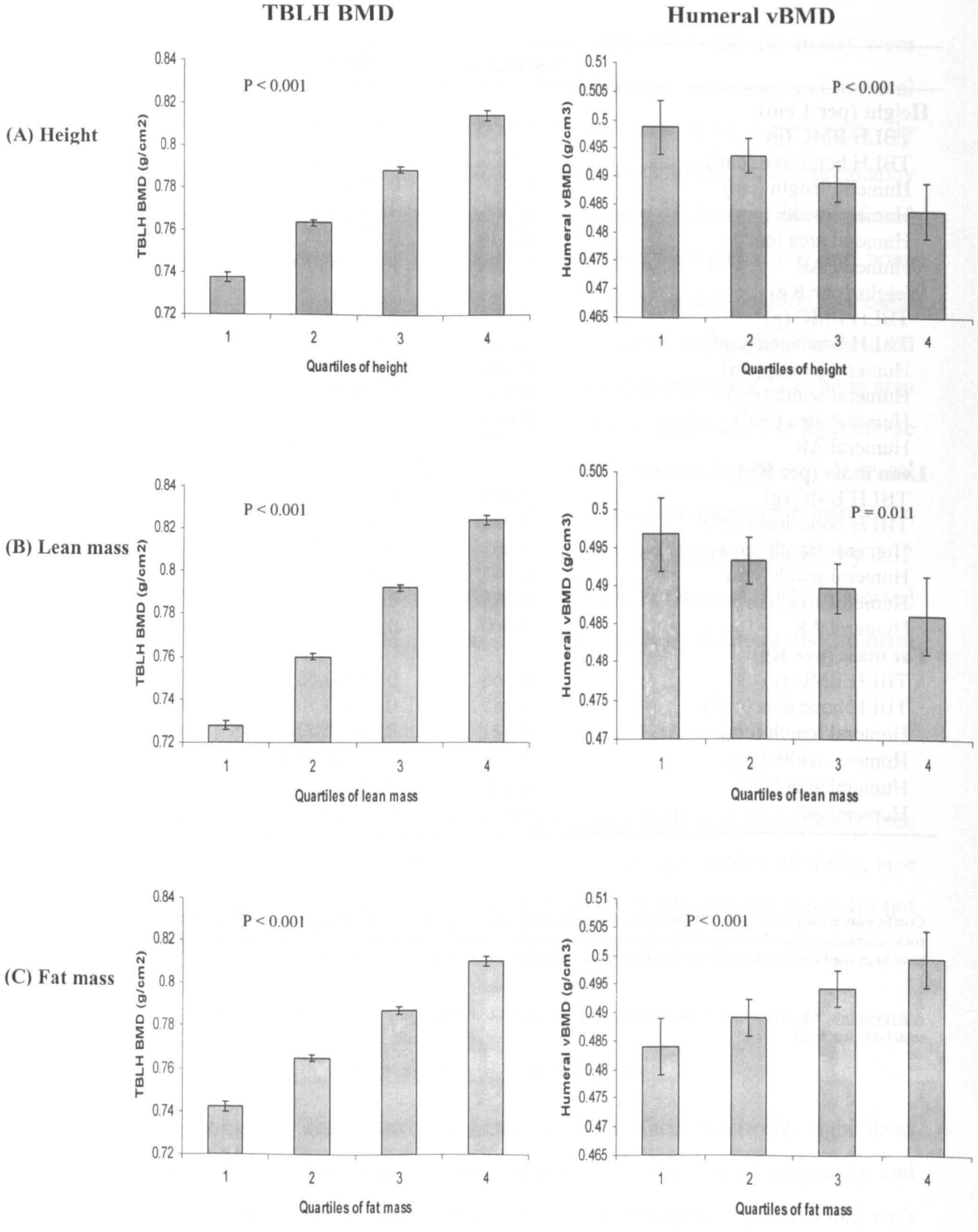
Table 54: Regression coefficients for the association between body composition and bone size in children aged 9.8 years. Coefficients are per Kg increase of body weight, fat and lean mass, and per 1 cm increase in height.

	Coefficient	95%CI	P value
Height (per 1 cm)			
TBLH BMC (g)	0.775	0.757, 0.793	< 0.001
TBLH bone area (cm^2)	0.827	0.811, 0.844	< 0.001
Humeral length (cm)	0.803	0.767, 0.840	< 0.001
Humeral width (cm)	0.557	0.507, 0.607	< 0.001
Humeral area (cm^2)	0.770	0.731, 0.809	< 0.001
Humeral AR	- 0.020	-0.079, 0.040	0.521
Weight (per Kg)			
TBLH BMC (g)	0.827	0.810, 0.843	< 0.001
TBLH bone area (cm^2)	0.849	0.833, 0.865	< 0.001
Humeral length (cm)	0.558	0.510, 0.606	< 0.001
Humeral width (cm)	0.519	0.469, 0.568	< 0.001
Humeral area (cm^2)	0.630	0.585, 0.675	< 0.001
Humeral AR	-0.150	-0.207, -0.092	< 0.001
Lean mass (per Kg)			
TBLH BMC (g)	0.898	0.882, 0.914	< 0.001
TBLH bone area (cm^2)	0.920	0.905, 0.935	< 0.001
Humeral length (cm)	0.685	0.636, 0.734	< 0.001
Humeral width (cm)	0.741	0.695, 0.788	< 0.001
Humeral area (cm^2)	0.845	0.805, 0.885	< 0.001
Humeral AR	-0.307	-0.362, -0.240	< 0.001
Fat mass (per Kg)			
TBLH BMC (g)	0.665	0.642, 0.687	< 0.001
TBLH bone area (cm^2)	0.685	0.662, 0.707	< 0.001
Humeral length (cm)	0.421	0.368, 0.474	< 0.001
Humeral width (cm)	0.339	0.283, 0.394	< 0.001
Humeral area (cm^2)	0.440	0.386, 0.493	< 0.001
Humeral AR	-0.055	-0.114, 0.004	0.069

Coefficients are adjusted for age at time of body composition and bone mass measurement, gender, ethnicity, size of pregnancy and socio-economic status (housing tenure, parental education and parental social class). To allow comparisons, standardised variables have been used for both the outcome (bone mass) and exposure variables (body composition)

Abbreviations: AR aspect ratio; BMC bone mineral content; CI confidence interval; cm centimetre; g grams; Kg kilogram; TBLH total body less head

Figure 62: Associations between body composition and estimated volumetric bone density at aged 9.8 years



Results show mean ± 95%CI and are adjusted for age, gender, ethnicity, size of pregnancy and socio-economic status (housing tenure, parental education and parental social class).

Abbreviations: BMC bone mineral content; BMD bone mineral density; cm centimetre; g grams; TBLH total body less head; vBMD volumetric bone density

12.4.4.4. Fat mass and bone mass

Fat mass and bone size

As shown in Table 54, page 279, fat mass is positively associated with bone size. To focus on this more closely, it was assessed whether the relationship found was independent of height and lean mass. The relationship between total body fat mass and TBLH bone area was attenuated, particularly when lean mass was included in the model (regression coefficient 0.283, 95%CI 0.268 to 0.298, P<0.001). Nevertheless, even when analyses were adjusted for both lean mass and height, a positive association between fat mass and TBLH bone area persisted (regression coefficient 0.274, 95%CI 0.261, 0.288, P<0.001). Similar results were obtained after adjusting for both height and height-squared (results not shown). Similar results were obtained after adjusting for pubertal stage. There was evidence for an interaction between fat mass and lean mass in terms of their effects on TBLH bone area (P<0.001), but inclusion of a fat mass-lean mass interaction parameter in the regression analyses did not affect the results.

Fat mass and bone size or mass at weight-bearing bones

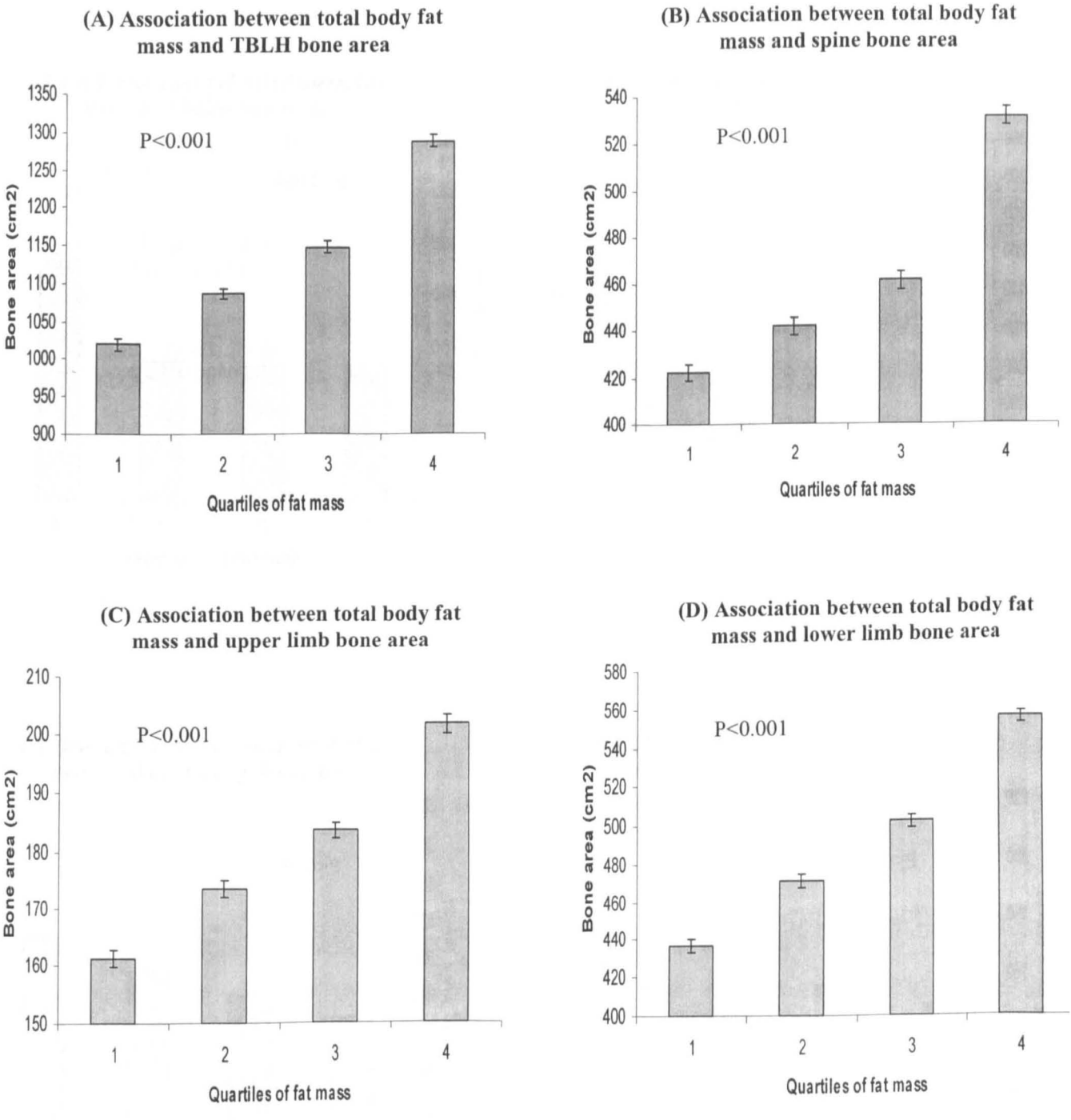
To determine whether fat mass preferentially influenced bone growth at weight bearing bones the association between total body fat mass and bone mass of the trunk (spine), upper limbs and lower limbs was assessed. When bone area was examined in relation to quartile of fat mass, an equivalent linear dose-response relationship was observed between fat mass and bone area of the spine, upper and lower limb to that seen for total body less head bone area (see Figure 63, page 283). Similar, although attenuated results were seen after adjustment for height and lean mass (see Figure 64, page 284). Similar results were seen for the relationship between total body fat mass and BMC or BMD of the spine, upper and lower limbs (results not shown).

Fat mass distribution and bone mass

To investigate whether fat mass distribution (i.e. trunkal or peripheral) influences bone mass, the effects of total body, trunkal, and peripheral (upper and lower limb combined) fat mass on TBLH bone mass were examined. After adjusting for age, gender, ethnicity, size of pregnancy and socio-economic status, a similar relationship was seen between peripheral fat mass and TBLH bone area to that between trunk fat mass and TBLH bone

area. See Table 55, page 285. Similar, although attenuated results, were seen after adjusting for height and lean mass.

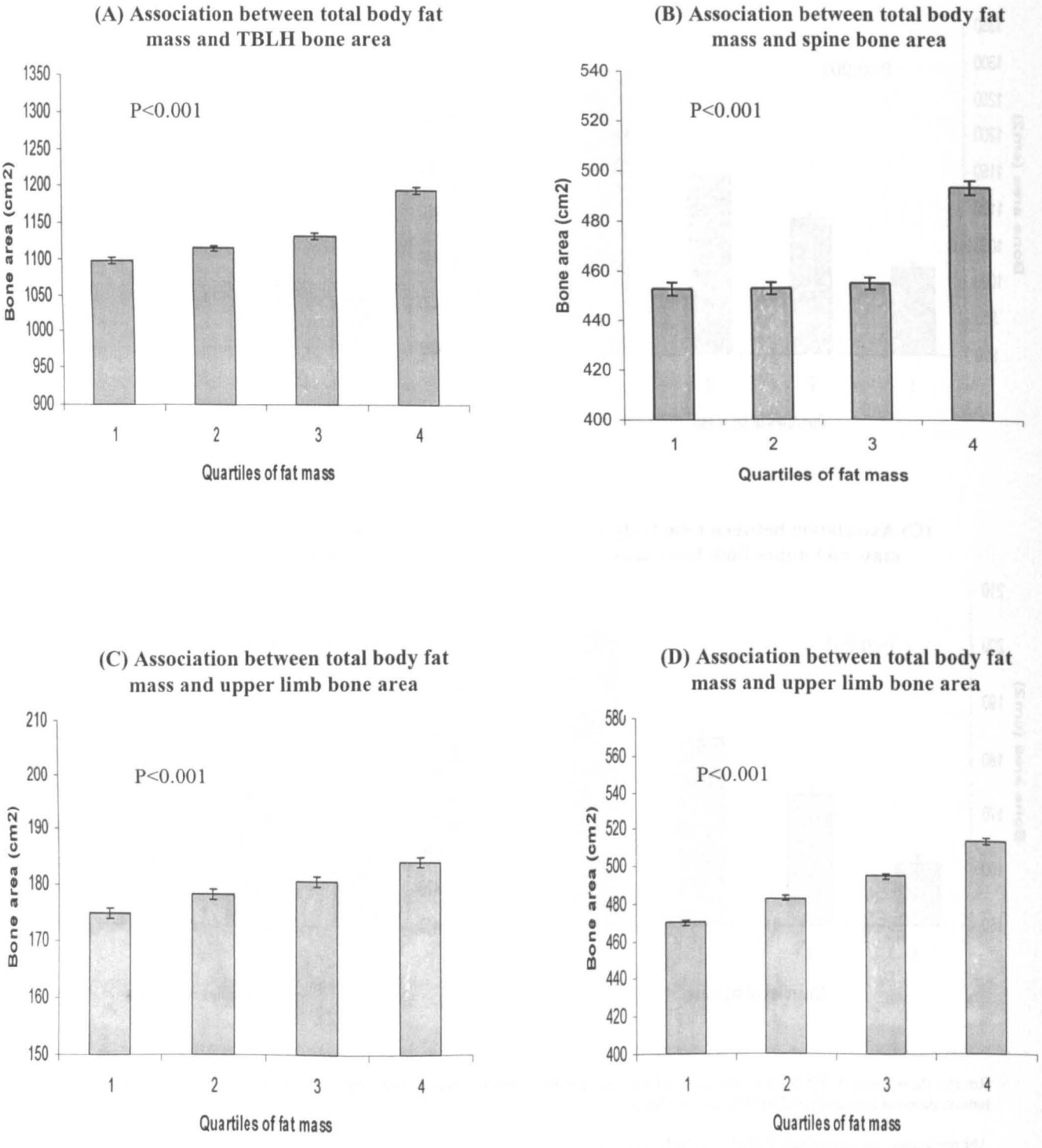
Figure 63: Effect of total body fat mass on skeletal area (A) Total body less head (B) spine (C) upper limb and (D) lower limb area.



Results show mean ± 95%CI and are adjusted for age, gender, ethnicity, size of pregnancy and socio-economic status (housing tenure, parental education and parental social class).

Abbreviations: cm centimetre; TBLH total body less head

Figure 64: Effect of total body fat mass on skeletal area (A) Total body less head (B) spine (C) upper limb and (D) lower limb area, adjusted for height and lean mass



Results show mean ± 95%CI and are adjusted for age, gender, ethnicity, size of pregnancy, socio-economic status (housing tenure, parental education and parental social class), height and lean mass.

Abbreviations: cm centimetre; TBLH total body less head

Table 55: Associations between fat mass at different sites and TBLH bone area

Regression model	N	Fat mass versus TBLH bone area at aged 9.8 years	
		Coefficient (95%CI)	P value
Model 1: Total body fat mass			
Boys and girls	4336	0.684 (0.662, 0.707)	< 0.001
Boys	2131	0.622 (0.589, 0.655)	< 0.001
Girls	2205	0.743 (0.712, 0.774)	< 0.001
Model 2 : Trunk fat mass			
Boys and girls together	4336	0.670 (0.647, 0.693)	< 0.001
Boys	2131	0.622 (0.587, 0.656)	< 0.001
Girls	2205	0.712 (0.681, 0.744)	< 0.001
Model 3: Peripheral fat mass			
Boys and girls together	4336	0.683 (0.661, 0.706)	< 0.001
Boys	2131	0.613 (0.581, 0.645)	< 0.001
Girls	2205	0.753 (0.722, 0.785)	< 0.001

Coefficients are per 1 kg increase in fat mass calculated by multivariable linear regression. Coefficients with 95% confidence intervals were calculated using standardised continuous variables (minus mean, divided by standard deviation) for fat mass at the different sites and bone area.

Abbreviations: CI confidence interval; Kg kilogram; TBLH total body less head

12.5. SUMMARY

12.5.1. Association between social position and body composition

These results show that lower social position is associated with a greater body weight, a greater fat mass, and a shorter overall height in primary school children, but there is no social gradient in lean mass. The differences in body weight, fat mass and height were modest, but these may have been reduced by the selective loss to follow-up amongst children from less advantaged backgrounds. The lack of a social gradient in lean mass may reflect the lack of a strong social gradient in physical activity in this ALSPAC cohort (503).

12.5.2. The association between social position and bone mass

At first sight, social position measured during pregnancy does not seem to be associated with bone mass of the offspring at aged 9.8 years. However, when the influence of height and weight were explored, the results suggest that effects of social position on height and weight exert important, but opposing influences on bone size and bone mineral content in childhood. In other words, children born to mothers from lower social positions were shorter but heavier, while children born to mothers from higher social positions were taller but lighter: consequently the bone size and bone mineral content in children from low or high social positions were similar.

After adjusting the association between social position and bone mass for height, the weight-dependent effect of social position on TBLH BMC or bone area must reflect an effect of weight on appositional bone growth, as this is the only mechanism apart from longitudinal bone growth whereby increases in the size of the skeletal envelope occur (see section on modelling, Literature Review, Chapter 2, page 28). So these observations suggest that, whereas social position exerts a positive influence on BMC and bone area as a consequence of effects on longitudinal bone growth, this is opposed by a negative effect on appositional bone growth caused by associated changes in fat mass. This was confirmed by the observation of no overall association between social position and estimated volumetric bone density.

12.5.3. The association between body composition and bone mass

All measures of body composition were positively associated with measures of bone size. Humeral aspect ratio (AR) was negatively associated with both weight and lean mass. This is consistent with the effects of increased mechanical loading by body weight or lean mass resulting in a bone less likely to fracture, as according to beam theory, columns with smaller aspect ratios (i.e. shorter and fatter) have a reduced fracture risk than columns with greater aspect ratios (25).

Height, lean mass and fat mass were positively associated with total body BMD, and fat mass was positively associated with estimated humeral volumetric density. Conversely, height and lean mass were negatively associated with humeral volumetric bone density. This negative association is most likely to be due to an over-correction of BMC for size, rather than representing a true relationship. i.e. given the problems of attempting to estimate volumetric density using adjusted DXA-derived measures, it is unclear whether this method can be used to examine the relationship between height and volumetric density. It is most likely that the 'true' association lies somewhere between that seen for the association between height and TBLH BMD, and that seen for height and estimated humeral volumetric density.

These are the first investigations into the influence of fat mass on bone mass, and they provide strong evidence that adipose tissue acts to stimulate both bone size and volumetric density in children. Since the relationship between fat mass and bone size in models which included lean mass was not affected by additional adjustment for height, these results suggest that fat mass acts to increase bone size by stimulating radial rather than longitudinal bone growth, presumably by increasing the rate of periosteal apposition. This is confirmed by the positive association between total body fat mass and width of the humerus. A preferential action of fat mass at weight-bearing sites was not seen, suggesting that mechanical load-bearing alone does not explain how adipose tissue might stimulate bone growth.

12.6. CONCLUSIONS

- Children from lower social positions have a greater body weight, a greater fat mass and a shorter overall height than children from higher social positions

- No overall association is seen between social position and volumetric bone density

- Social position exerts two opposing influences on bone size acquisition in childhood
    - On the one hand, social position tends to increase bone size in childhood, as a consequence of a positive association with longitudinal growth.
    - On the other hand, although children born to mothers from a lower social position were shorter, their bone size seemed to be preserved as a consequence of their greater fat mass

- Height and lean mass are positive predictors of both bone size and volumetric bone density

- Weight and lean mass influence humeral geometry to produce a bone that is less likely to fracture (reduced AR).

- Fat mass is an important positive independent determinant of periosteal bone formation in prepubertal children

- Fat mass is an important positive independent determinant of volumetric bone density

CHAPTER 13: THE ASSOCIATION BETWEEN CHILD, MATERNAL, PATERNAL AND SOCIO-ECONOMIC DATA WITH FRACTURES IN CHILDREN BETWEEN AGES 9.8 AND 11.7 YEARS

The previous results chapters described the variables used in the Fracture Study (Chapter 11) and explored the confounding structure of childhood bone mass (Chapter 12). This chapter presents the associations between child data (excluding bone mass), maternal data, paternal data and socio-economic data with fractures between ages 9.8 and 11.7 years. The next chapter presents the association between bone mass measured at aged 9.8 years and fracture risk over the following two year (Chapter 14).

13.1. INTRODUCTION

The literature on the determinants of fractures in childhood is limited mainly to small studies, usually case control in design. This has been reviewed in detail in Chapter 5, section 2, but is briefly summarised here. Despite this paucity of large population-based studies, there is consistent evidence that boys more commonly fracture than girls (see Literature Review, Chapter 5, page 125), and that there is an association between cola beverage intake and fracture risk (see Literature Review, Chapter 5, page 138) although it is possible that cola drink consumption is a behavioural marker rather than a casual factor *per se*.

There is contradictory evidence on the association between ethnicity and childhood fractures (see Literature Review, Chapter 5, Page 126) and no literature is available on the association between early life factors such as gestational age, birth weight or breast feeding and fracture risk. Small single studies have investigated associations between psychological status (see Literature Review, Chapter 5, page 137) such as risk taking behaviour or ADHD and fracture risk in childhood. The evidence for an association between physical activity and fracture risk in childhood is confused by poorly defined measures such as time spent watching TV (Literature Review, Chapter 5, page 140)

which is more a measure of sedentariness, or participation in sporting activities without defining which activities. There is contradictory evidence for the association between dietary intake such as calcium and fracture risk in childhood (Literature Review, Chapter 5, page 138), and a single study looked at the association with puberty (Literature Review, Chapter 5, page 133). No conclusions can be drawn from the literature on the association between obesity (Literature Review, Chapter 5, page 135), or socio-economic status (Literature Review, Chapter 5, page 141) and fracture risk in childhood because of contradictory small studies. No literature is available on the association between maternal or paternal factors and childhood fracture risk.

13.2. AIMS

The aims of this chapter are to use a population-based birth cohort study to investigate fracture risk in childhood, specifically to

1. Confirm the association between gender and fracture risk between ages 9.8 and 11.7 years.

2. Provide further data on the association between ethnicity, psychological status, physical activity, diet, puberty, anthropometry and socio-economic status and fracture risk between ages 9.8 and 11.7 years.

3. Provide the first evidence for the association between early life factors, maternal factors or paternal factors and fracture risk between ages 9.8 and 11.7 years.

13.3. METHODS

13.3.1. Study population

As already described in the previous results chapter (see page 262), the study population is described by Figure 28, Methods, Chapter 9, page 214, and consists of 5933 children from ALSPAC, a birth cohort study (see Method, Chapter 8, page 179). The inclusion criteria for the Fracture Study were that the children had attended a research clinic at

aged 9.8 years for a DXA scan, and further research clinics at aged 10.7 years and also at aged 11.7 years. Ethical approval for this study had obtained from the ALSPAC Law and Ethics Committee and from three LRECs (see Methods, Chapter 9, page 202).

13.3.2. Exposure variables

A summary of exposure variables used for this chapter are shown in tabular form on page 196 in Methods, Chapter 8. All of these variables have been collected prior to the 2-year period when fractures (the outcome) were recorded. The methods of data collection for each variable are discussed in Methods, Chapter 8 pages 184 to 195. The distribution of the variables, simple gender associations and social gradients for the exposure variables are illustrated in Results, Chapter 11 starting on page 227 along with information on missing data.

The variables used in this chapter have been divided into

1. Child data
    (a) demographic data: gender and ethnicity
    (b) early life data: gestational age, birth weight, breast feeding
    (c) psychological status: risk taking behaviour, ADHD, OCD
    (d) physical activity data: balance, locomotor ability, weekly vigorous physical activity, time spent watching TV, time spent in a vehicle, time spent outdoors in summer and winter
    (e) dietary data: daily calcium, vitamin D and total energy intake
    (f) pubertal status: girls Tanner staging for breast and pubic hair development, boys Tanner staging for pubic hair development
    (g) anthropometry: height, weight and BMI at aged 9.8 years
2. Maternal data
    (a) size of pregnancy
    (b) maternal smoking during pregnancy
    (c) maternal body size pre-pregnancy: height, weight and BMI
    (d) maternal history of fractures: upper and lower limb
    (e) maternal age at delivery

3. Paternal data

(a) paternal body size: height, weight and BMI

(b) paternal history of fractures: upper and lower limb

4. Family data

(a) family size

5. Socio-economic data

(a) housing tenure

(b) parental education: maternal and paternal

(c) parental social class: maternal and paternal

13.3.3. Outcome variable: fractures

Data were collected on reported fractures occurring in this study population in the 2 years since their DXA scan at aged 9.8 years. The method of data collection is described in Methods, Chapter 9, page 214. Where written X-ray reports were available, 82% of reported fractures were confirmed (see Methods, Chapter 9, page 216). For the rest of this chapter it is reported fractures that are used as the outcome, not verified fractures, because less than 50% of reported fractures had X-ray reports available. Children were classified as having a reported fracture if they reported one or more than one fracture in the 2-year follow-up period. Currently, no data is available on the date of fracture, the age of the child at fracture or details of the injury resulting in the fracture.

13.3.4. Statistical analysis

All statistical analyses were performed with Stata 8.0 (see Methods, Chapter 10, page 219). The outcome measure was presence or absence of reported fracture over the two-year period as a binary outcome. Odds of exposure to the putative risk factors in those with fractures compared to those without fractures were calculated.

Odds ratios were used to assess the measure of effect of the various factors on fracture risk. These were calculated using the Mantel-Haenszel method (see Methods, Chapter 10, page 225). Crude ORs were calculated for comparison with the baseline category. The Mantel-Haenszel test for trend was also calculated by treating the categorical variables as continuous in the analysis. Stratified analyses were then carried out to look

for evidence of effect modification or interaction (see Methods, Chapter 10, page 226). Multivariable logistic regression was used to investigate the effect of multiple putative risk factors on the outcome of fractures during aged 9.8 to 12 years.

Analyses of early life data were performed unadjusted, and then always adjusted for gestational age used as a continuous variable. Analyses of dietary data were performed unadjusted, and then always adjusted for total energy intake as a continuous variable. Analyses of pubertal data were performed unadjusted, and then always adjusted for age at time of pubertal measurement used as a continuous variable. Analyses of anthropometrical data were performed unadjusted, and then always adjusted for age at measurement, used as a continuous variable. All analyses were additionally adjusted for socio-economic data. All analyses were additionally adjusted for size of pregnancy (see Methods, Chapter 11, page 245).

To look for independent determinants of fracture risk, all variables found to be associated with fractures during minimally adjusted analyses (defined as $P<0.05$) were included in a multivariable regression model.

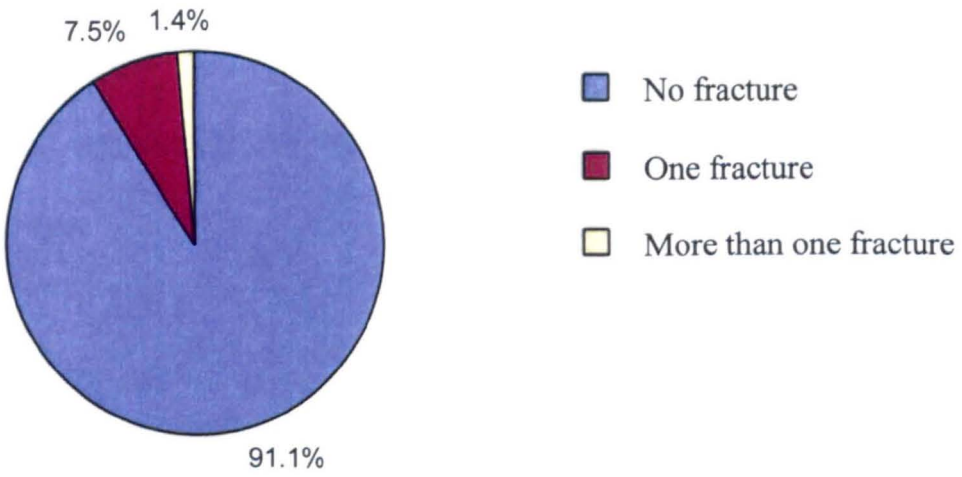
13.4. RESULTS

13.4.1. Basic description of the study population

Of the 5993 children in this Fracture Study, 2901 (48.9%) were male and 191 (3.5%) were non-white (see Results Chapter 11, page 227). Comparison with the rest of the ALSPAC cohort shows preferential loss of male children and those of non-white ethnicity (see Results Chapter 11, page 256).

Of the 5933 children 527 (8.9%) reported at least one fracture in the previous two years: 443 (7.5%) reported one fracture; and 84 (1.4%) reported more than one fracture. See Figure 65 below.

Figure 65: Number of children with none, one or more than one fracture over the two-year follow-up period



13.4.2. Association between demographics and fractures

Crude ORs for the association between gender and fractures between age 9.8 and 11.7 show an increased risk of fractures in boys (OR 1.24, 95%CI 1.03 to 1.48, P=0.021) compared to girls. In unadjusted analyses (crude) there was a reduced risk of fractures in children from non-white ethnicity compared to white ethnicity (OR 0.40, 95%CI 0.19 to 0.85, P=0.018), however, this was based on just seven fractures in non-white children. See Table 56, page 297. No change in these measures of effect for gender and ethnicity was seen after adjustment for each other, or after adjustment for social background (housing tenure, parental education and parental social class). Gender and ethnicity were included in all further analyses as potential confounding factors.

13.4.3. Association between early life data and fractures

Crude odds ratio for the association between any fracture between age 9.8 and 11.7 years and early life variables (see Table 56, page 297) showed a reduced risk of fractures in children of low birth weight compared to those of normal birth weight (OR 0.55, 95%CI 0.32 to 0.96, P=0.034). No other univariable associations were seen. Stratification of ORs for the association between birth weight and fractures into gender revealed evidence of interaction: in boys the OR was 0.26 (95%CI 0.08 to 0.90) but for girls was 0.71 (95%CI 0.26 to 1.97), P=0.075. There was no pre-specified hypothesis that this interaction was likely, as no previous literature has found this interaction and there is no obvious biological mechanism. Therefore it was considered likely to be due to chance and results for boys and girls were presented together. Adjusting the association between birth weight and fractures for height and weight at aged 9.8 years did not affect the OR estimate (OR= 0.52, 95%CI 0.25 to 1.09). There was no other evidence of interaction or effect modification.

Adjustment for gestational age, social background, and ethnicity did not change the crude estimates. Using birth weight as a continuous variable in the regression analyses, per 250g increase in birth weight the risk for fractures adjusted for gestational age, size of pregnancy, social background, gender and ethnicity increased by 9% (OR 1.09,

95%CI 1.02 to 1.15, P=0.008). Use of gestational age as a continuous variable confirmed the null association found in Table 56, page 297.

Table 56: Association between demographics and early life factors with fractures between aged 9.8 and 11.7 years. The total number of fractures is 527.

	Fracture		No fracture		Crude OR	Adjusted OR (A)	Adjusted OR (B)	Adjusted OR (C)	Adjusted OR (C)
	N	%	N	%	OR 95% CI, P value	OR 95% CI	OR 95%CI	OR 95%CI	OR 95%CI
Gender									
Female	244	46.3	2788	51.6	1.0	1.0	1.0	1.0	1.0
Male	283	53.7	2618	48.4	1.24 (1.03, 1.48) P=0.021	1.24 (1.03, 1.50)	1.24 (1.03, 1.50)	1.30 (1.05, 1.61)	1.26 (1.01, 1.57)
Ethnicity									
White	455	98.5	4764	96.3	1.0	1.0	1.0	1.0	1.0
Non-white	7	1.5	184	3.7	0.40 (0.19, 0.85) P=0.018	0.40 (0.19, 0.85)	0.40 (0.19, 0.85)	0.33 (0.12, 0.90)	0.26 (0.08, 0.81)
Gestational age									
≥ 37 weeks	468	94.9	4861	94.4	1.0		1.0	1.0	1.0
preterm delivery	25	5.1	291	5.6	0.89 (0.59, 1.36) P=0.594		0.89 (0.57, 1.37)	0.98 (0.60, 1.62)	1.22 (0.69, 2.13)
Birth weight									
normal (≥ 2500g)	475	97.1	4832	95.0	1.0	1.0	1.0	1.0	1.0
low (< 2500g)	14	2.9	257	5.0	0.55 (0.32, 0.96) P=0.034	0.47 (0.26, 0.86)	0.45 (0.24, 0.84)	0.51 (0.24, 1.06)	0.47 (0.21, 1.01)
Breast feeding									
never	95	20.5	828	17.3	1.0	1.0	1.0	1.0	1.0
< 1 month	68	14.7	753	15.7	0.79 (0.57, 1.09)	0.79 (0.57, 1.09)	0.79 (0.57, 1.09)	0.87 (0.60, 1.27)	0.86 (0.59, 1.26)
1-3 months	69	14.9	761	15.9	0.79 (0.57, 1.09)	0.79 (0.57, 1.09)	0.79 (0.57, 1.09)	0.86 (0.59, 1.26)	0.85 (0.58, 1.25)
3-6 months	68	14.7	687	14.3	0.86 (0.57, 1.20)	0.86 (0.62, 1.20)	0.86 (0.62, 1.20)	0.99 (0.68, 1.46)	1.01 (0.68, 1.48)
>6 months	164	35.3	1770	36.9	0.81 (0.62, 1.05)	0.81 (0.62, 1.05)	0.81 (0.62, 1.06)	0.91 (0.65, 1.27)	0.90 (0.64, 1.25)
					OR Test for Trend: 0.97 (0.91, 1.03) P=0.273	***OR Test for Trend:*** 0.97 (0.91, 1.03)	***OR Test for Trend:*** 0.97 (0.91, 1.03)	***OR Test for Trend:*** 0.99 (0.92, 1.07)	***OR Test for Trend:*** 0.99 (0.92, 1.07)

(A) ORs adjusted for gestational age (used as a continuous variable)
(B) ORs adjusted for size of pregnancy and gestational age
(C) ORs adjusted for socio-economic status (housing tenure, parental education, parental social class), size of pregnancy and gestational age
(D) ORs adjusted for all other variables in the table, socio-economic status, size of pregnancy and gestational age

Abbreviations: CI confidence interval; OR odds ratio

13.4.4. Association between psychological status at aged 7.6 years and fractures from aged 9.8 to 11.7 years

No clear evidence of an association between ADHD, OCD or risk avoidance and fractures between age 9.8 and 11.7 was found. However, there was a suggestion that the presence of a diagnosis of ADHD or OCD increased the risk of fractures, as the 95% confidence intervals were mainly above 1.0 e.g. OR for ADHD compared with no diagnosis of ADHD of 1.72, 95%CI 0.93 to 3.18. See Table 57, page 299. Adjustment for size of pregnancy, social background, gender or ethnicity did not change the lack of association. There was no evidence of effect modification or interaction.

13.4.5. Association between physical activity at aged 4.5 years and 9 years with fractures from aged 9.8 to 11.7 years

Crude ORs showed an increased risk of fractures in those children who spend more than 28 hours outdoors per week in summer (OR 1.33, 95%CI 1.04 to 1.68, P=0.021), in those who do more vigorous physical activity (OR Test for Trend 1.39, 95%CI 1.18 to 1.62, P<0.001) and in those with the best locomotor ability at aged 4.5 years (OR 1.18, 95%CI 1.05 to 1.33, P=0.006). See Table 58, page 300. There was no other evidence of interaction or effect modification. As with all previous analyses, adjusting for size of pregnancy did not change the results. However, adjusting for social background reduced the OR for time spent outdoors in summer from 1.33 to 1.17. As this is a greater than 10% change it is suggestive that social background confounds the association between time spent outdoors during summer and fractures in children. Adjusting for social background, gender and ethnicity did not change any of the other ORs, apart from to suggest that spending more time outside in the winter may be protective for fractures (adjusted OR Test for Trend 0.87, 95%CI 0.76 to 0.99). Use of time spent watching TV, time spent in a vehicle, time spent outdoors in summer or winter, number of correct steps and locomotor ability as continuous variables confirmed the associations found in Table 58, starting on page 300.

Table 57: Association between psychological status measured at 7.6 years with fractures between aged 9.8 and 11.7 years.

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI
ADHD													
No	412	97.2	4478	98.3	1.0			1.0		1.0		1.0	
Yes	12	2.8	76	1.7	1.72	(0.93, 3.18)	P=0.086	1.72	(0.93, 3.18)	2.04	(1.02, 4.06)	1.90	(0.95, 3.82)
OCD													
No	406	95.8	4432	97.3	1.0			1.0		1.0		1.0	
Yes	18	4.3	122	2.7	1.61	(0.97, 2.67)	P=0.064	1.61	(0.97, 2.67)	1.59	(0.86, 2.95)	1.49	(0.80, 2.79)
Risk avoidance													
Never or hardly ever	216	48.0	2022	42.7	1.0			1.0		1.0		1.0	
Sometimes	162	36.0	1970	41.6	0.77	(0.62, 0.95)		0.77	(0.62, 0.95)	0.82	(0.64, 1.04)	0.83	(0.65, 1.05)
Often or very often	72	16.0	740	15.6	0.91	(0.69, 1.20)		0.91	(0.69, 1.21)	0.95	(0.69, 1.29)	0.99	(0.72, 1.35)
					OR Test for Trend:			***OR Test for Trend:***		***OR Test for Trend:***		***OR Test for Trend:***	
					0.91	(0.79, 1.04)	P=0.165	0.91	(0.79, 1.04)	0.94	(0.80, 1.09)	0.95	(0.82, 1.11)

(A) Adjusted for size of pregnancy
(B) Adjusted for socio-economic status (housing tenure, parental education and parental social class) and size of pregnancy
(C) Adjusted for gender, ethnicity, socio-economic status and size of pregnancy

Abbreviations: ADHD attention deficit hyperactive disorder; CI confidence interval; OCD oppositional/conduct disorder; OR odds ratio

Table 58: Associations between physical activity at aged 4.5 years and 9 years with fractures between aged 9.8 and 11.7 years.

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI
Time spent watching TV (tertiles)													
1 (least)	122	29.0	1250	27.5	1.0			1.0		1.0		1.0	
2	152	36.1	1954	43.0	0.80 (0.62, 1.02)			0.80 (0.62, 1.02)		0.77 (0.58, 1.02)		0.76 (0.57, 1.01)	
3 (most)	147	34.9	1344	29.6	1.12 (0.87, 1.44)			1.12 (0.87, 1.44)		1.01 (0.75, 1.36)		0.99 (0.74, 1.34)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.07 (0.94, 1.22) P=0.317			1.07 (0.94, 1.22)		1.01 (0.87, 1.18)		1.00 (0.86, 1.17)	
Time spent in a vehicle (tertiles)													
1 (least)	56	13.3	495	10.9	1.0			1.0		1.0		1.0	
2	223	53.1	2525	55.6	0.78 (0.57, 1.06)			0.78 (0.57, 1.06)		0.81 (0.57, 1.16)		0.84 (0.58, 1.20)	
3 (most)	141	33.6	1518	33.5	0.82 (0.59, 1.14)			0.82 (0.59, 1.14)		0.81 (0.55, 1.19)		0.84 (0.58, 1.24)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.94 (0.81, 1.11) P=0.473			0.94 (0.81, 1.11)		0.93 (0.77, 1.11)		0.94 (0.79, 1.13)	
Time spent outdoors in summer per week													
0-28 hours	93	22.2	1247	27.4	1.0			1.0		1.0		1.0	
more than 28 hours	326	77.8	3298	72.6	1.33 (1.04, 1.68) P=0.021			1.33 (1.04, 1.68)		1.17 (0.90, 1.53)		1.15 (0.88, 1.50)	
Time spent outdoors in winter (tertiles)													
1 (least)	113	27.0	1085	24.0	1.0			1.0		1.0		1.0	
2	100	23.9	1053	23.3	0.91 (0.69, 1.21)			0.91 (0.69, 1.21)		0.92 (0.67, 1.25)		0.93 (0.68, 1.27)	
3 (most)	205	49.1	2391	52.8	0.82 (0.65, 1.05)			0.82 (0.65, 1.05)		0.77 (0.59, 1.25)		0.76 (0.58, 1.00)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.91 (0.81, 1.02) P=0.107			0.91 (0.81, 1.02)		0.88 (0.77, 1.00)		0.87 (0.76, 0.99)	
Weekly vigorous physical activity													
less than 4 episodes	120	44.0	1869	56.5	1.0			1.0		1.0		1.0	
4-6 episodes	97	35.5	965	29.2	1.57 (1.19, 2.07)			1.57 (1.19, 2.07)		1.74 (1.26, 2.39)		1.72 (1.25, 2.37)	
daily	56	20.5	473	14.3	1.84 (1.32, 2.57)			1.86 (1.33, 2.59)		2.03 (1.39, 2.97)		2.01 (1.36, 2.97)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.39 (1.18, 1.62) P<0.001			1.39 (1.19, 1.63)		1.46 (1.22, 1.75)		1.45 (1.21, 1.75)	

Table 58, continued

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI
No of correct steps in total (tertiles)													
1 (least)	60	14.4	597	13.5	1.0			1.0		1.0		1.0	
2	131	31.3	1540	34.9	0.85 (0.62, 1.17)			0.84 (0.61, 1.16)		0.76 (0.53, 1.08)		0.78 (0.55, 1.12)	
3 (most)	227	54.3	2282	51.6	0.99 (0.73, 1.33)			0.99 (0.73, 1.33)		0.75 (0.53, 1.05)		0.79 (0.56, 1.11)	
					OR Test for Trend: 1.04 (0.90, 1.20) P=0.618			*OR Test for Trend:* 1.04 (0.90, 1.19)		*OR Test for Trend:* 0.89 (0.76, 1.05)		*OR Test for Trend:* 0.91 (0.77, 1.08)	
No of steps before an error (tertiles)													
1 (least)	96	23.0	941	21.3	1.0			1.0		1.0		1.0	
2	95	22.7	1196	27.1	0.78 (0.58, 1.05)			0.78 (0.58, 1.05)		0.78 (0.56, 1.08)		0.78 (0.56, 1.09)	
3 (most)	227	54.3	2282	51.6	0.98 (0.76, 1.25)			0.97 (0.76, 1.26)		0.80 (0.60, 1.08)		0.82 (0.62, 1.10)	
					OR Test for Trend: 1.02 (0.90, 1.15) P=0.808			*OR Test for Trend:* 1.02 (0.90, 1.15)		*OR Test for Trend:* 0.90 (0.78, 1.04)		*OR Test for Trend:* 0.92 (0.79, 1.06)	
Locomotor ability (tertiles)													
1 (least)	154	36.8	1958	42.9	1.0			1.0		1.0		1.0	
2	119	28.4	1277	28.0	1.18 (0.92, 1.52)			1.18 (0.92, 1.52)		1.18 (0.90, 1.55)		1.21 (0.92, 1.59)	
3 (most)	146	34.8	1332	29.7	1.39 (1.10, 1.77)			1.39 (1.10, 1.77)		1.22 (0.93, 1.60)		1.29 (0.98, 1.70)	
					OR Test for Trend: 1.18 (1.05, 1.33) P=0.006			*OR Test for Trend:* 1.18 (1.05, 1.33)		*OR Test for Trend:* 1.11 (0.97, 1.27)		*OR Test for Trend:* 1.14 (0.99, 1.31)	

(A) Adjusted for size of pregnancy
(B) Adjusted for socio-economic status (housing tenure, parental education and parental social class) and size of pregnancy
(C) Adjusted for gender, ethnicity, socio-economic status and size of pregnancy

Abbreviations: CI confidence interval; OR odds ratio; TV television

13.4.6. Association between diet at aged 6.8 years and fractures from aged 9.8 to 11.7 years

Crude ORs show an increased risk of fractures in those children who have a greater total energy intake: OR Test for Trend 1.11 (95%CI 1.01 to 1.21), see Table 59, page 303. No association was seen between calcium or vitamin D intake and fractures, even after adjustment for total energy intake. Adjustment for size of pregnancy, social background, gender or ethnicity did not change any of the crude ORs. There was no evidence of effect modification or interaction. Use of total energy intake, calcium intake or vitamin D intake (log transformed, see Results, Chapter 11, page 234) as continuous variables confirmed the associations found in Table 59, page 303.

13.4.7. Association between pubertal status at aged 9 years and fractures from aged 9.8 to 11.7 years

No association was seen between any of the measures of pubertal status and fractures, see Table 60, page 304. Adjustment for age at measurement of pubertal status, size of pregnancy, social background or ethnicity did not change any of the crude ORs. There was no evidence of effect modification or interaction. However, these results were based on small numbers of children in Tanner stages 2 or more with fractures.

13.4.8. Association between anthropometry or body composition at aged 9.8 years and fractures from aged 9.8 to 11.7 years

No association was seen between any of the anthropometric measures and fractures, see Table 61, page 305. Adjustment for age at measurement, size of pregnancy, social background, gender or ethnicity did not change any of the crude ORs. There was no evidence of effect modification or interaction. Use of height, weight (1/square-root transformed, see Results, Chapter 11, page 243) or BMI (inverse transformed, see results, Chapter 11, page 244) as continuous variables confirmed the null associations found in Table 61, page 305.

Table 59: Associations between diet at aged 6.8 years and fractures between aged 9.8 and 11.7 years

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)		Adjusted OR (D)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Total energy intake															
1 (lowest)	92	22.0	1145	25.3	1.0					1.0		1.0		1.0	
2	103	24.6	1133	25.0	1.13 (0.84, 1.52)					1.13 (0.84, 1.52)		1.12 (0.80, 1.56)		1.08 (0.78, 1.51)	
3	96	23.0	1141	25.2	1.05 (0.78, 1.41)					1.05 (0.78, 1.41)		1.06 (0.76, 1.48)		1.04 (0.75, 1.45)	
4 (highest)	127	30.4	1109	24.5	1.43 (1.08, 1.89)					1.43 (1.08, 1.89)		1.39 (1.01, 1.91)		1.35 (0.98, 1.86)	
					OR Test for Trend:					*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.11 (1.01, 1.21) P=0.025					1.11 (1.01, 1.21)		1.10 (0.99, 1.22)		1.09 (0.99, 1.21)	
Calcium intake															
1 (lowest)	107	25.6	1130	25.0	1.0			1.0		1.0		1.0		1.0	
2	86	20.6	1150	25.4	0.79 (0.59, 1.06)			0.73 (0.54, 0.99)		0.73 (0.54, 0.99)		0.77 (0.54, 1.08)		0.77 (0.55, 1.09)	
3	101	24.2	1136	25.1	0.94 (0.71, 1.25)			0.83 (0.60, 1.13)		0.83 (0.60, 1.13)		0.89 (0.62, 1.26)		0.89 (0.63, 1.26)	
4 (highest)	124	29.6	1112	24.6	1.18 (0.90, 1.55)			0.93 (0.64, 1.35)		0.93 (0.64, 1.35)		0.84 (0.55, 1.29)		0.84 (0.55, 1.29)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.07 (0.98, 1.17) P=0.131			0.99 (0.87, 1.12)		0.99 (0.87, 1.12)		0.96 (0.84, 1.10)		0.96 (0.84, 1.10)	
Vitamin D intake															
1 (lowest)	100	23.9	1137	25.1	1.0			1.0		1.0		1.0		1.0	
2	95	22.7	1141	25.2	0.95 (0.71, 1.27)			0.91 (0.67, 1.22)		0.91 (0.67, 1.22)		0.93 (0.67, 1.31)		0.94 (0.67, 1.32)	
3	112	26.8	1125	24.9	1.13 (0.85, 1.50)			1.04 (0.77, 1.39)		1.04 (0.77, 1.39)		1.07 (0.77, 1.49)		1.06 (0.76, 1.48)	
4 (highest)	111	26.6	1125	24.8	1.12 (0.85, 1.49)			0.96 (0.70, 1.31)		0.96 (0.70, 1.31)		1.15 (0.81, 1.64)		1.16 (0.81, 1.66)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.05 (0.96, 1.15) P=0.252			1.00 (0.90, 1.11)		1.00 (0.90, 1.11)		1.06 (0.94, 1.19)		1.06 (0.94, 1.19)	

(A) adjusted for total energy intake (continuous variable): calcium and vitamin D only
(B) adjusted for size of pregnancy and total energy intake
(C) adjusted for socio-economic status (housing tenure, parental education, parental social class) size of pregnancy and total energy intake
(D) adjusted for gender, ethnicity, socio-economic status, size of pregnancy and total energy intake

Abbreviations: CI confidence interval; OR odds ratio

Table 60: Associations between pubertal stage at aged 9 and fractures between age 9.8 and 11.7 years

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)		Adjusted OR (D)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Girls breast stage															
pre-pubertal	124	66.3	1431	63.0	1.0			1.0		1.0		1.0		1.0	
Tanner stage 2	56	30.0	705	31.0	0.92 (0.66, 1.27)			0.91 (0.66, 1.27)		0.91 (0.66, 1.27)		0.85 (0.57, 1.27)		0.86 (0.58, 1.28)	
Tanner stage 3 or more	7	3.7	137	6.0	0.59 (0.27, 1.29)			0.59 (0.27, 1.29)		0.58 (0.27, 1.29)		0.37 (0.12, 1.21)		0.37 (0.12, 1.20)	
					OR Test for Trend:			***OR Test for Trend:***		***OR Test for Trend:***		***OR Test for Trend:***		***OR Test for Trend:***	
					0.85 (0.66, 1.10)		P=0.218	0.85 (0.65, 1.10)		0.85 (0.65, 1.10)		0.76 (0.55, 1.05)		0.76 (0.55, 1.06)	
Girls pubic hair stage															
pre-pubertal	155	82.5	1869	81.9	1.0			1.0		1.0		1.0		1.0	
Tanner stage 2	29	15.4	334	14.6	1.05 (0.69, 1.59)			1.04 (0.69, 1.57)		1.04 (0.68, 1.56)		0.81 (0.48, 1.37)		0.82 (0.48, 1.39)	
Tanner stage 3 or more	4	2.1	80	3.5	0.60 (0.22, 1.67)			0.60 (0.22, 1.65)		0.58 (0.21, 1.62)		0.64 (0.20, 2.09)		0.70 (0.21, 2.32)	
					OR Test for Trend:			***OR Test for Trend:***		***OR Test for Trend:***		***OR Test for Trend:***		***OR Test for Trend:***	
					0.92 (0.67, 1.26)		P=0.596	0.91 (0.66, 1.25)		0.90 (0.66, 1.24)		0.81 (0.54, 1.21)		0.83 (0.55, 1.25)	
Boys pubic hair stage															
pre-pubertal	152	82.2	1523	82.2	1.0			1.0		1.0		1.0		1.0	
Tanner stage 2,3,4 or 5	33	17.8	330	17.8	1.00 (0.68, 1.49)		P=0.992	1.00 (0.68, 1.49)		1.01 (0.68, 1.49)		0.91 (0.57, 1.44)		0.91 (0.58, 1.45)	

(A) adjusted for age at puberty measure (continuous variable)
(B) adjusted for size of pregnancy and age at puberty measure
(C) adjusted for socio-economic status (housing tenure, parental education, parental social class) size of pregnancy and age at puberty measure
(D) adjusted for ethnicity, socio-economic status, size of pregnancy and age at puberty measure

Abbreviations: CI confidence interval; OR odds ratio

Table 61: Association between anthropometry at aged 9.8 years and fractures between age 9.8 and 11.7 years

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)		Adjusted OR (D)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Height: quartiles															
1 (lowest)	118	22.4	1392	25.8	1.0			1.0		1.0		1.0		1.0	
2	142	26.9	1319	24.4	1.27 (0.98, 1.64)			1.26 (0.97, 1.63)		1.26 (0.97, 1.63)		1.31 (0.97, 1.78)		1.30 (0.96, 1.78)	
3	147	27.9	1349	25.0	1.29 (0.99, 1.66)			1.26 (0.97, 1.63)		1.26 (0.98, 1.63)		1.29 (0.95, 1.75)		1.29 (0.95, 1.75)	
4 (highest)	120	22.8	1346	24.9	1.05 (0.81, 1.37)			1.01 (0.77, 1.33)		1.02 (0.78, 1.33)		1.02 (0.74, 1.41)		1.00 (0.72, 1.38)	
					OR Test for Trend: 1.02 (0.94, 1.10), P=0.689			*OR Test for Trend:* 1.01 (0.93, 1.09)		*OR Test for Trend:* 1.01 (0.93, 1.09)		*OR Test for Trend:* 1.00 (0.91, 1.11)		*OR Test for Trend:* 0.99 (0.90, 1.10)	
Weight: quartiles															
1 (lowest)	132	25.1	1371	25.4	1.0			1.0		1.0		1.0		1.0	
2	148	28.1	1384	25.6	1.11 (0.87, 1.42)			1.12 (0.88, 1.44)		1.12 (0.88, 1.44)		1.25 (0.93, 1.68)		1.22 (0.91, 1.64)	
3	141	26.8	1320	24.4	1.11 (0.87, 1.42)			1.13 (0.88, 1.45)		1.13 (0.88, 1.45)		1.15 (0.85, 1.56)		1.12 (0.82, 1.52)	
4 (highest)	106	20.1	1331	24.6	0.83 (0.63, 1.08)			0.85 (0.65, 1.11)		0.85 (0.65, 1.11)		0.93 (0.67, 1.28)		0.92 (0.67, 1.27)	
					OR Test for Trend: 0.95 (0.87, 1.03), P=0.211			*OR Test for Trend:* 0.96 (0.88, 1.04)		*OR Test for Trend:* 0.96 (0.88, 1.04)		*OR Test for Trend:* 0.97 (0.88, 1.07)		*OR Test for Trend:* 0.97 (0.88, 1.07)	
BMI: quartiles															
1 (lowest)	115	21.8	1369	25.3	1.0			1.0		1.0		1.0		1.0	
2	143	27.1	1340	24.8	1.27 (0.98, 1.64)			1.26 (0.98, 1.63)		1.26 (0.98, 1.63)		1.21 (0.90, 1.63)		1.19 (0.88, 1.61)	
3	129	24.5	1354	25.1	1.13 (0.87, 1.47)			1.13 (0.87, 1.46)		1.13 (0.87, 1.47)		1.09 (0.81, 1.48)		1.09 (0.80, 1.48)	
4 (highest)	140	26.6	1343	24.8	1.24 (0.96, 1.61)			1.22 (0.95, 1.59)		1.23 (0.95, 1.59)		1.13 (0.83, 1.54)		1.16 (0.85, 1.59)	
					OR Test for Trend: 1.05 (0.97, 1.14), P=0.211			*OR Test for Trend:* 1.05 (0.97, 1.14)		*OR Test for Trend:* 1.05 (0.97, 1.14)		*OR Test for Trend:* 1.03 (0.93, 1.13)		*OR Test for Trend:* 1.04 (0.94, 1.14)	

(A) adjusted for age at measurement (continuous variable)
(B) adjusted for size of pregnancy and age at measurement
(C) adjusted for socio-economic status (housing tenure, parental education, parental social class) size of pregnancy and age at measurement
(D) adjusted for gender, ethnicity, socio-economic status, size of pregnancy and age at measurement

Abbreviations: CI confidence interval; OR odds ratio

13.4.9. Associations between maternal factors and fractures in offspring aged 9.8 to 11.7 years

Crude ORs show an increased risk of fractures in those children whose mothers have a history of leg fracture (OR 1.41, 95%CI 1.00 to 1.97, P=0.048), and an inverse association between maternal age and fractures in the offspring (OR Test for Trend 0.87, 95%CI 0.77 to 0.97, P=0.014). There was a suggestion that maternal smoking during pregnancy increased the risk of fractures, as the 95% confidence intervals were mainly above 1.0 e.g. OR for mothers who smoked compared with those who did not of 1.20, 95%CI 0.92 to 1.57. See Table 62, page 308. No association was seen between any other maternal factor and fractures.

Adjusting for social background reduced the OR for maternal smoking from 1.20 to 1.05. As this is a greater than 10% change it is suggestive that social background confounds the association between maternal smoking during pregnancy and fractures in children. Adjustment for size of pregnancy, social background, gender or ethnicity did not change any of the other crude ORs. There was no evidence of effect modification or interaction. Use of maternal smoking, maternal height, maternal weight and maternal BMI (log transformed, see Results, Chapter 11, page 247) as continuous variables confirmed the associations found in Table 62, page 308. Use of maternal age as a continuous variable shows that per 5-year increase in maternal age at delivery, the unadjusted risk of fractures in the offspring reduces by 13% (OR Test for Trend 0.87, 95%CI 0.78 to 0.97, P=0.009). However, when using maternal age as a continuous variable, adjustment for social background changes this OR from 0.87 to 0.96 (95%CI 0.84 to 1.10, P=0.575). As this is a greater than 10% change it is suggestive that social background confounds the association between maternal age and fractures in children.

13.4.10. Associations between paternal factors and fractures in offspring aged 9.8 to 11.7 years

No association was seen between any of the paternal factors and fractures, see Table 63, page 310. Adjustment for size of pregnancy, social background, gender or ethnicity did not change any of the crude ORs. There was no evidence of effect modification or

interaction. Use of paternal height, paternal weight or paternal BMI as continuous variables confirmed the null associations found in Table 63, page 310.

Table 62: Associations between maternal factors and fractures in offspring aged 9.8 to 11.7 years

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI
Size of pregnancy													
Singleton	513	97.3	5275	97.6	1.0					1.0		1.0	
Twin or triplet	14	2.7	131	2.4	1.10 (0.63, 1.92)		P=0.741			0.75 (0.35, 1.64)		0.74 (0.34, 1.61)	
Maternal smoking during pregnancy													
No	413	85.9	4452	88.0	1.0			1.0		1.0		1.0	
Yes	68	14.1	610	12.0	1.20 (0.92, 1.57)		P=0.183	1.20 (0.92, 1.57)		1.05 (0.75, 1.48)		1.04 (0.74, 1.47)	
Maternal pre-pregnancy height: tertiles													
1 (shortest)	171	36.1	1607	32.5	1.0			1.0		1.0		1.0	
2	134	28.3	1479	29.9	0.85 (0.67, 1.08)			0.85 (0.67, 1.08)		0.90 (0.69, 1.17)		0.91 (0.70, 1.19)	
3 (tallest)	169	35.6	1865	37.7	0.85 (0.68, 1.06)			0.85 (0.68, 1.06)		0.90 (0.70, 1.15)		0.90 (0.70, 1.16)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.92 (0.82, 1.04)		P=0.162	0.92 (0.83, 1.03)		0.95 (0.84, 1.08)		0.95 (0.84, 1.08)	
Maternal pre-pregnancy weight: tertiles													
1 (lightest)	181	40.0	1878	39.5	1.0			1.0		1.0		1.0	
2	111	24.6	1370	28.8	0.84 (0.66, 1.08)			0.84 (0.66, 1.08)		0.86 (0.56, 1.13)		0.85 (0.65, 1.12)	
3 (heaviest)	160	35.4	1505	31.7	1.10 (0.88, 1.38)			1.10 (0.88, 1.38)		1.07 (0.83, 1.38)		1.06 (0.82, 1.36)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.05 (0.93, 1.17)		P=0.440	1.05 (0.93, 1.17)		1.03 (0.91, 1.17)		1.02 (0.90, 1.17)	
Maternal pre-pregnancy BMI: tertiles													
1 (lightest)	136	30.3	1586	33.6	1.0			1.0		1.0		1.0	
2	150	33.4	1574	33.4	1.11 (0.87, 1.42)			1.11 (0.87, 1.42)		1.14 (0.87, 1.48)		1.14 (0.87, 1.49)	
3 (heaviest)	163	36.3	1556	33.0	1.22 (0.96, 1.55)			1.22 (0.96, 1.55)		1.19 (0.91, 1.55)		1.19 (0.91, 1.56)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.11 (0.98, 1.25)		P=0.099	1.11 (0.98, 1.25)		1.09 (0.95, 1.24)		1.09 (0.95, 1.25)	

Table 62, continued

	Fracture N	Fracture %	No fracture N	No fracture %	Crude OR OR	95% CI	P value	Adjusted OR (A) OR	95%CI	Adjusted OR (B) OR	95%CI	Adjusted OR (C) OR	95%CI
Maternal history of arm fractures													
No	396	83.5	4263	85.7	1.0			1.0		1.0		1.0	
Yes	78	16.5	712	14.3	1.18 (0.91, 1.52)		P=0.206	1.18 (0.91, 1.52)		1.15 (0.86, 1.53)		1.14 (0.86, 1.52)	
Maternal history of leg fractures													
No	434	91.2	4660	93.6	1.0			1.0		1.0		1.0	
Yes	42	8.8	321	6.4	1.41 (1.00, 1.97)		P=0.048	1.40 (1.00, 1.97)		1.38 (0.94, 2.02)		1.38 (0.94, 2.03)	
Maternal age at delivery: tertiles													
1 (youngest)	195	39.6	1808	35.1	1.0			1.0		1.0		1.0	
2	174	35.3	1797	34.9	0.90 (0.72, 1.11)			0.90 (0.72, 1.11)		0.94 (0.73, 1.20)		0.95 (0.74, 1.22)	
3 (oldest)	124	25.1	1547	30.0	0.74 (0.59, 0.94)			0.74 (0.59, 0.94)		0.85 (0.64, 1.12)		0.86 (0.65, 1.13)	
					OR Test for Trend: 0.87 (0.77, 0.97)		P=0.014	***OR Test for Trend:*** 0.86 (0.77, 0.97)		***OR Test for Trend:*** 0.92 (0.80, 1.06)		***OR Test for Trend:*** 0.93 (0.81, 1.07)	

(A) adjusted for size of pregnancy
(B) adjusted for socio-economic status (housing tenure, parental education, parental social class) and size of pregnancy
(C) adjusted for gender of child, ethnicity, socio-economic status and size of pregnancy

Abbreviations: BMI body mass index; CI confidence interval; OR odds ratio

Table 63: Association between paternal factors and fractures in offspring aged 9.8 to 11.7 years

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI
Paternal height: tertiles													
1 (shortest)	112	32.2	1192	31.8	1.0			1.0		1.0		1.0	
2	106	30.5	1191	31.7	0.95 (0.72, 1.25)			0.95 (0.72, 1.25)		0.99 (0.72, 1.36)		0.99 (0.72, 1.36)	
3 (tallest)	130	37.4	1370	36.5	1.01 (0.78, 1.32)			1.01 (0.77, 1.31)		1.04 (0.77, 1.41)		1.03 (0.76, 1.39)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.01 (0.88, 1.15)		P=0.926	1.01 (0.88, 1.15)		1.02 (0.88, 1.19)		1.02 (0.88, 1.18)	
Paternal weight: tertiles													
1 (lightest)	139	40.2	1464	39.1	1.0			1.0		1.0		1.0	
2	113	32.7	1264	33.8	0.94 (0.73, 1.22)			0.94 (0.73, 1.22)		0.93 (0.70, 1.25)		0.92 (0.69, 1.23)	
3 (heaviest)	94	27.2	1017	27.2	0.97 (0.74, 1.28)			0.97 (0.74, 1.28)		0.97 (0.71, 1.32)		0.95 (0.70, 1.29)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.98 (0.86, 1.13)		P=0.813	0.98 (0.86, 1.13)		0.98 (0.84, 1.14)		0.97 (0.83, 1.13)	
Paternal BMI: tertiles													
1 (lightest)	122	35.3	1260	33.9	1.0			1.0		1.0		1.0	
2	112	32.4	1230	33.1	0.94 (0.72, 1.23)			0.94 (0.72, 1.23)		0.97 (0.71, 1.31)		0.96 (0.71, 1.31)	
3 (heaviest)	112	32.4	1229	33.0	0.94 (0.72, 1.23)			0.94 (0.72, 1.23)		1.01 (0.75, 1.36)		1.00 (0.74, 1.35)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.97 (0.85, 1.11)		P=0.655	0.97 (0.85, 1.11)		1.00 (0.86, 1.17)		1.00 (0.86, 1.16)	
Paternal history of arm fractures													
No	247	71.4	2801	75.1	1.0			1.0		1.0		1.0	
Yes	99	28.6	929	24.9	1.21 (0.95, 1.54)		P=0.129	1.21 (0.94, 1.54)		1.15 (0.87, 1.51)		1.15 (0.87, 1.51)	
Paternal history of leg fractures													
No	287	82.7	3152	84.5	1.0			1.0		1.0		1.0	
Yes	60	17.3	579	15.5	1.14 (0.85, 1.52)		P=0.385	1.14 (0.85, 1.53)		1.16 (0.84, 1.62)		1.15 (0.83, 1.60)	

(A) adjusted for size of pregnancy
(B) adjusted for socio-economic status (housing tenure, parental education, parental social class) and size of pregnancy
(C) adjusted for ethnicity, socio-economic status and size of pregnancy

Abbreviations: BMI body mass index; CI confidence interval; OR odds ratio

13.4.11. Associations between family and socio-economic factors and fractures from aged 9.8 to 11.7 years

Crude ORs show an increased risk of fractures in those children whose family is larger (OR Test for Trend 1.18, 95%CI 1.01 to 1.37, P=0.033), in those who live in rented accommodation (OR Test for Trend 1.17, 95%CI 1.01 to 1.36, P=0.043) and in those children whose fathers were from a lower social class (OR Test for Trend 1.08, 95%CI 1.00 to 1.16, P=0.048). There is a reduced risk of fractures in those children whose fathers have a higher education (OR Test for Trend 0.93, 95%CI 0.87 to 0.99, P=0.039). See Table 64, page 312. No association was seen between any other family or socio-economic factor and fractures.

Adjustment for size of pregnancy, all other socio-economic factors, gender or ethnicity did not change the association between family size and fractures. Adjustment for size of pregnancy, all other socio-economic factors, gender or ethnicity reduced the ORs for the association between housing tenure, paternal social class and paternal education towards the null. Adjustment of the association between housing tenure and fracture risk for family size attenuated the association completely. Adjustment of the association between paternal education or paternal social class and fracture risk for family size did not affect the association. There was no evidence of effect modification or interaction.

13.4.12. Independent determinants of fracture risk in children

To investigate whether any of the variables were independent predictors of fracture risk in children, a model was run containing all the child, maternal, paternal, family and socio-economic factors that were found on unadjusted analysis (crude) to be associated with fractures (see methods section for this chapter on page 293). Results showed that time spent outdoors in summer at aged 4.5 years (OR Test for Trend 1.62, 95%CI 1.09 to 2.40, P=0.009), locomotor ability at aged 6.8 years (OR Test for Trend 1.25, 95%CI 1.04 to 1.50, P=0.018), and the amount of vigorous physical activity per week at aged 9 years (OR Test for Trend 1.40, 95%CI 1.14 to 1.71, P=0.001) were independent predictors of fracture risk in children aged 9.8 to 11.7 years. Birthweight was not an independent predictor of childhood fractures.

Table 64: Association between family and socio-economic factors and fractures in children aged 9.8 to 11.7 years

	Fracture		No fracture		Crude OR			Adjusted OR (A)		Adjusted OR (B)		Adjusted OR (C)	
	N	%	N	%	OR	95% CI	P value	OR	95%CI	OR	95%CI	OR	95%CI
Family size													
1 to 3 members	77	17.5	780	16.9	1.0			1.0		1.0		1.0	
4 members	218	49.7	2675	57.9	0.83 (0.63, 1.08)			0.83 (0.63, 1.09)		0.99 (0.72, 1.38)		0.96 (0.70, 1.33)	
more than 4 members	144	32.8	1166	25.2	1.25 (0.94, 1.67)			1.26 (0.94, 1.69)		1.54 (1.08, 2.18)		1.48 (1.04, 2.10)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.18 (1.01, 1.37)		P=0.033	1.18 (1.02, 1.38)		1.29 (1.08, 1.55)		1.28 (1.07, 1.53)	
Housing tenure													
mortgaged/owned	390	83.3	4244	86.9	1.0			1.0		1.0		1.0	
private rental	30	6.4	240	4.9	1.36 (0.92, 2.02)			1.36 (0.92, 2.02)		1.52 (0.96, 2.42)		1.53 (0.96, 2.43)	
council rental / HA	48	10.3	398	8.2	1.31 (0.96, 1.80)			1.31 (0.96, 1.80)		1.05 (0.65, 1.70)		1.11 (0.69, 1.81)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					1.17 (1.01, 1.36)		P=0.043	1.17 (1.01, 1.36)		1.10 (0.88, 1.36)		1.12 (0.90, 1.40)	
Maternal education													
none/CSEs	64	13.5	611	12.2	1.0			1.0		1.0		1.0	
vocational	44	9.3	396	7.9	1.06 (0.71, 1.59)			1.06 (0.71, 1.59)		1.09 (0.66, 1.82)		1.12 (0.67, 1.87)	
O level	172	36.3	1764	35.2	0.93 (0.69, 1.26)			0.93 (0.69, 1.26)		0.94 (0.63, 1.40)		0.97 (0.65, 1.45)	
A level	120	25.3	1400	27.9	0.82 (0.60, 1.13)			0.82 (0.60, 1.12)		0.84 (0.54, 1.32)		0.88 (0.56, 1.38)	
degree	74	15.6	841	16.8	0.84 (0.59, 1.19)			0.84 (0.59, 1.19)		1.02 (0.61, 1.72)		1.07 (0.63, 1.80)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.94 (0.87, 1.02)		P=0.120	0.94 (0.87, 1.02)		0.98 (0.86, 1.10)		0.99 (0.87, 1.11)	
Paternal education													
none/CSEs	99	21.5	911	18.6	1.0			1.0		1.0		1.0	
vocational	46	10.0	382	7.8	1.11 (0.77, 1.60)			1.11 (0.77, 1.60)		0.99 (0.63, 1.57)		0.99 (0.63, 1.56)	
O level	93	20.2	1075	22.0	0.80 (0.59, 1.07)			0.80 (0.59, 1.07)		0.78 (0.53, 1.13)		0.77 (0.53, 1.12)	
A level	131	28.4	1402	28.7	0.86 (0.65, 1.13)			0.86 (0.65, 1.13)		0.98 (0.69, 1.40)		0.98 (0.68, 1.40)	
degree	92	20.0	1122	22.9	0.76 (0.56, 1.02)			0.75 (0.56, 1.01)		0.84 (0.54, 1.31)		0.83 (0.53, 1.30)	
					OR Test for Trend:			*OR Test for Trend:*		*OR Test for Trend:*		*OR Test for Trend:*	
					0.93 (0.87, 0.99)		P=0.039	0.93 (0.87, 0.99)		0.98 (0.88, 1.08)		0.98 (0.88, 1.08)	

Table 64, continued

	Fracture N	Fracture %	No fracture N	No fracture %	Crude OR OR	Crude OR 95% CI	Crude OR P value	Adjusted OR (A) OR	Adjusted OR (A) 95%CI	Adjusted OR (B) OR	Adjusted OR (B) 95%CI	Adjusted OR (C) OR	Adjusted OR (C) 95%CI
Maternal social class													
I	33	8.1	303	6.9	1.0			1.0		1.0		1.0	
II	136	33.4	1561	35.7	0.80 (0.54, 1.19)			0.80 (0.54, 1.19)		0.75 (0.48, 1.18)		0.76 (0.48, 1.19)	
IIInm	157	38.6	1850	42.3	0.80 (0.53, 1.16)			0.78 (0.53, 1.16)		0.74 (0.46, 1.20)		0.74 (0.46, 1.20)	
IIIm	34	8.4	279	6.4	1.12 (0.68, 1.86)			1.13 (0.68, 1.88)		1.03 (0.56, 1.88)		0.99 (0.54, 1.83)	
IV	40	9.8	332	7.6	1.11 (0.68, 1.80)			1.11 (0.68, 1.81)		0.97 (0.52, 1.78)		0.97 (0.53, 1.80)	
V	7	1.7	52	1.2	1.24 (0.52, 2.94)			1.23 (0.52, 2.92)		1.52 (0.57, 4.04)		1.56 (0.58, 4.18)	
					OR Test for Trend: 1.08 (0.98, 1.18) P=0.138			*OR Test for Trend:* 1.08 (0.98, 1.19)		*OR Test for Trend:* 1.08 (0.95, 1.22)		*OR Test for Trend:* 1.08 (0.95, 1.22)	
Paternal social class													
I	53	12.1	608	13.1	1.0			1.0		1.0		1.0	
II	150	34.3	1715	36.9	1.00 (0.72, 1.39)			1.00 (0.72, 1.39)		0.94 (0.65, 1.37)		0.94 (0.65, 1.36)	
IIInm	44	10.1	571	12.3	0.88 (0.58, 1.34)			0.88 (0.58, 1.34)		0.88 (0.55, 1.41)		0.89 (0.55, 1.42)	
IIIm	136	31.1	1283	27.6	1.22 (0.87, 1.69)			1.22 (0.87, 1.70)		1.16 (0.76, 1.75)		1.15 (0.76, 1.73)	
IV	47	10.7	367	7.9	1.47 (0.97, 2.22)			1.48 (0.98, 2.24)		1.39 (0.83, 2.32)		1.41 (0.84, 2.36)	
V	8	1.8	100	2.2	0.92 (0.42, 1.99)			0.92 (0.43, 1.99)		0.50 (0.17, 1.48)		0.50 (0.17, 1.49)	
					OR Test for Trend: 1.08 (1.00, 1.16) P=0.048			*OR Test for Trend:* 1.08 (1.00, 1.16)		*OR Test for Trend:* 1.05 (0.95, 1.17)		*OR Test for Trend:* 1.06 (0.95, 1.17)	

(A) adjusted for size of pregnancy
(B) adjusted for all other variables in the table and size of pregnancy
(C) adjusted for gender, ethnicity, all other variables in the table and size of pregnancy

Abbreviations: CI confidence interval; HA housing association; m manual; nm non-manual; OR odds ratio

13.5. SUMMARY

These results confirm that boys have an approximate 24% increased risk of fracture compared with girls aged 9.8 to 11.7 years.

These results clarify the association between ethnicity, psychological status, physical activity, diet, puberty, anthropometry and socio-economic status and fracture risk between ages 9.8 to 11.7 years. The association between ethnicity and fracture needs to be interpreted with caution as it was based on only 7 fractures in non-white children. No association was found between psychological status, puberty or anthropometry and fracture risk. Vigorous physical activity, locomotor ability and time spent outside during the summer were positive independent predictors of fracture risk in children. Total energy intake was also positively associated with fracture risk, perhaps as if the child is in energy balance then energy intake is a measure of energy expenditure and thus physical activity, but calcium or vitamin D intake were not associated. Children from lower socio-economic backgrounds measured by paternal education or paternal social class, had an increased risk of fracture, as did children from larger families.

These results provide the first evidence for the association between early life factors, maternal factors or paternal factors and fracture risk between ages 9.8 to 11.7 years. Children born of low birth weight had an approximate 50% reduced risk of fracture compared with children of normal birth weight. This association was still present after adjusting for body size (height and weight) at aged 9 years suggesting that it may be foetal biology i.e. prenatal factors rather than post-natal growth that are important in this association between low birth weight and fractures (504). There was a 41% increased risk of fractures in those children whose mothers had a history of leg fracture. Socio-economic status provided an alternative explanation (i.e. was a confounder) for the association seen between maternal smoking or maternal age and fractures. No other early life, maternal or paternal factors were associated with fracture risk in the children aged 9.8 to 11.7 years.

13.5.1. Conclusions

Risk factors for childhood fractures found in this study can be divided into three main groups. These are not mutually exclusive, and one risk factor may be present in more than one group:

- Those that could act via bone fragility
    - low socio-economic status (paternal education and social class)
    - larger families
    - maternal history of leg fracture
- Those that could increase the number of injuries a child experiences
    - high levels of vigorous physical activity at aged 9 years
    - increased time spent outdoors in summer at aged 7.6 years
    - low socio-economic status (paternal education and social class)
    - larger families
    - higher birth weight
- Others
    - male gender
    - white ethnicity
    - increased total energy intake at aged 6.8 years
    - better locomotor ability at aged 4.5 years

CHAPTER 14: THE ASSOCIATION BETWEEN BONE MASS MEASURED AT AGED 9.8 YEARS AND FRACTURE RISK OVER THE FOLLOWING TWO YEARS

The previous results chapters have described the distribution of variables used in the Fracture Study (Chapter 11), the confounding structure of bone mass (Chapter 12) and the early life and other determinants of fracture risk (Chapter 13). This last results chapter explores the association between bone mass (estimated volumetric density and bone size) measured at aged 9.8 years and fracture risk over the following two years. A paper has been published in a peer review journal based on this chapter (see Appendix L page 445).

14.1. INTRODUCTION

Fractures in children are common and increasing (see Literature Review, Chapter 5, page 122). Understanding the aetiology of fractures in children may provide opportunities for developing population-based interventions aimed at reversing this increase.

It is well-known that low bone mass or osteoporosis increases fracture risk in adults (see Literature Review, Chapter 2, page 41), but the evidence for this association in children is less established. The systematic review and meta-analysis of previous studies investigating the association between bone mass and fractures in children is discussed in depth in the Literature Review, Chapter 6, page 147. Briefly, this found evidence for an association between low bone mass and fractures in children with an SMD of -0.32 (95%CI -0.43 to -0.21, P<0.001), but this needs to be interpreted with caution as it is based on eight case control studies, as no prospective studies had been carried out.

Bone mass as reflected by DXA-derived measures of BMC is affected by both overall bone size and by volumetric bone density i.e. the amount of bone mineral per unit

volume (see Literature Review, Chapter 2, page 48), but these previous case control studies have not distinguished between bone size or volumetric density. Volumetric density is in turn influenced by cortical thickness, cortical porosity, trabecular bone volume and the level of tissue mineralisation (see Literature Review, Chapter 2, page 32). Biomechanical strength indices such as cross-sectional moment of inertia (CSMI, see Literature Review, Chapter 2, page 38) depend on both bone size (cortical width) and bone density (particularly cortical thickness), but CSMI has not been investigated as a risk factor for childhood fractures.

One explanation for the lack of differentiation between bone size, bone density and biomechanical strength indices when assessing fracture risk in children is the difficulty in estimating these parameters from total body DXA scans. However, various methods have been developed for estimating volumetric bone density from DXA measurements based on more complete adjustment of BMC for bone size (505), suggesting that it may be possible to distinguish the contributions of bone size and volumetric bone density to fracture risk using DXA-derived data.

14.2. AIMS OF THIS CHAPTER

The aim of this chapter is to describe the first prospective study of the association between bone mass as measured by DXA and fractures in children using a large population-based cohort, specifically to

1. Investigate the association between estimated volumetric bone density measured at aged 9.8 and fracture risk over the following 2 years
2. Investigate the association between bone size measured at aged 9.8 and fracture risk over the following 2 years
3. Investigate the association between biomechanical strength indices measured at aged 9.8 and fracture risk over the following 2 years
4. Finally, to assess whether the other risk factors for childhood fracture risk found in Chapter 13 (see page 315 for a summary) act independently of volumetric bone density, bone size or bone biomechanical strength indices.

14.3. METHODS

14.3.1. Study population

As discussed previously in Results Chapters 12 (page 262) and Chapter 13 (page 290) the study population is described by Figure 28, page 214 in Methods, Chapter 9, and consists of 5933 children from ALSPAC, a birth cohort study (see Methods Chapter 8, page 179). The inclusion criteria for the Fracture Study were that the children had attended a research clinic at aged 9.8 years for a DXA scan, and further research clinics at aged 10.7 years and also at aged 11.7 years. Ethical approval for this study had obtained from the ALSPAC Law and Ethics Committee and from three LRECs (see Methods Chapter 9, page 202).

14.3.2. Measures of estimated volumetric bone density

The measures of estimated volumetric bone density were the same as in Results, Chapter 12, page, 263. TBLH BMC was measured using a Lunar Prodigy DXA scanner at aged 9.8 years. The methods of data collection have been described in Methods, Chapter 8, page 189. TBLH BMC adjusted for body size (height, weight, and TBLH bone area), was used to estimate total body volumetric density. From the regional humeral analyses, estimated volumetric density (humeral vBMD) was calculated by assuming the humerus was a cylinder and dividing humeral BMC by humeral volume derived by the following equation:

Humeral volume = humeral length X humeral cross-sectional area (Πr^2)

where r was calculated as half the humeral width.

14.3.3. Measures of bone size

The measures of bone size were the same as in Results, Chapter 12, page 262. TBLH bone area was measured using a Lunar Prodigy DXA scanner at aged 9.8 years. The method of data collection have been described in Methods Chapter 8, page 189. A sub-

sample of children had regional analyses of the humerus performed (see Methods Chapter 9, page 203) and the following measures of bone size were used in this chapter: humerus length, humerus width, humerus area, and humeral aspect ratio. Humeral aspect ratio (AR) was calculated as length divided by width.

14.3.4. Measures of biomechanical strength indices

Humeral CSMI and relative bone strength were calculated from the regional analyses of the humerus, using equations obtained from personal communication with TJ Beck and available in reference (506). CSMI was calculated by using the equation

$$CSMI = \frac{\Pi\ (r_o^4 - r_i^4)}{4}$$

Where r_o (radius of outer cortex) was half the width, and r_i (radius of inner cortex) was calculated as

$$\sqrt{r_o^2 - f_c \frac{CSA}{\pi}}$$

where f_c is the fraction of the bone mass in the cortex, taken as 1 for diaphyseal regions such as the shaft of the humerus.

Humeral CSA (excluding soft tissue spaces) was calculated as average thickness (BMD divided by the average mineral density of 1.052g/cm^3) multiplied by the humeral width. It is recognised that this arbitrary constant of 1.052g/cm^3 is derived from adult values and may not be valid in children.

Section modulus (Z) was calculated as CSMI divided by r_o. Z was divided by one half the length of the humerus as a proxy for moment arm length to obtain a measure of geometric strength index. This was normalised to an estimate of muscle index as suggested by the American Society for Bone and Mineral Research (ASBMR) Pediatric Bone Initiative (507), taken as upper limb lean mass measured by the total body DXA scan, and called relative bone strength.

14.3.5. Outcome variable: fractures

This is the same outcome measure used in Results, Chapter 13, page 292. Data were collected on reported fractures occurring in this study population in the 2 years since their DXA scan at aged 9.8 years. The method of data collection is described in Methods, Chapter 9, page 214. Where written X-ray reports were available, 82% of reported fractures were confirmed (see Methods, Chapter 9, page 216). For the rest of this chapter it is reported fractures that are used as the outcome, not verified fractures, as already discussed in Results, Chapter 13, page 292. Children were classified as having a reported fracture if they reported one or more than one fracture in the 2-year follow-up period.

14.3.6. Other measures

Age at the time of the DXA scan was recorded (see Methods, Chapter 8, page 189). Data on gender (see Methods, Chapter 8, page 184), ethnicity (see Methods, Chapter 8, page 184), size of pregnancy (see Methods, Chapter 8, page 192) and pubertal status (see Methods, Chapter 8, page 189) had been previously collected. The measures of social position used in this chapter were housing tenure, parental education and parental social class. The methods of data collection have been described in Methods, Chapter 8, page 194. The basic distribution of the measures of social position are described in Results, Chapter 11, page 253. For the purposes of this chapter maternal education has been coded 1 for no formal qualifications or CSEs, 2 for vocational qualifications, 3 for O levels, 4 for A levels and 5 for degree. For this chapter the measures of body composition used were height and weight at aged 9.8 years, and fat and lean mass from the DXA scans. The methods of data collection have been described in Methods, Chapter 8, page 191.

14.3.7. Statistical analyses

All statistical analyses were performed with Stata 8.0 (see Methods, Chapter 10, page 219). The outcome measure was presence or absence of reported fracture over the two-year period as a binary outcome. Odds of exposure to the putative risk factors in those with fractures compared to those without fractures were calculated.

14.3.7.1. Analysing the association between estimated volumetric bone density and fractures

Logistic regression was used to calculate ORs and 95% CIs to describe the association between estimated volumetric bone density and the risk of fractures over the following two years (see Methods, Chapter 10, page 225). Z scores were used to standardise an individuals estimated volumetric bone density (see Methods, Chapter 10, page 221) so ORs are for fracture risk per SD unit change. Z scores were produced for the continuous variables TBLH BMC, TBLH BA, height and weight by subtracting from the mean and dividing by the SD. The analyses were initially run adjusted for age at DXA measurement, and then progressively adjusted for gender, ethnicity, size of pregnancy and socio-economic status (housing tenure, parental education and parental social class). Analyses were also further adjusted for fat and lean mass. To assess whether any of the risk factors for childhood fracture found in Chapter 13 ((vigorous physical activity, locomotor ability, time spent outdoors in summer, total energy intake, family size, birth weight or maternal history of leg fracture) could be explained by an effect on estimated volumetric density, these were added to the regression in a step-wise manner to see if any independent association remained. Participants with missing data were excluded from analysis. Stratified analyses were then carried out to look for evidence of effect modification or interaction (see Methods, Chapter 10, page 226). Interactions between variables were assessed by including a multiplicative interaction term in the regression models and calculating the likelihood ratio test (LRT).

14.3.7.2. Analysing the association between bone size and fractures

Logistic regression was used to calculate ORs and 95% CIs to describe the association between bone size and the risk of fractures over the following two years (see Methods, Chapter 10, page 225). Z scores were used to standardise an individuals bone size (see Methods, Chapter 10, page 221) so ORs are for fracture risk per SD unit change. The analyses were initially run adjusted for age at DXA measurement, and then progressively adjusted for gender, ethnicity, size of pregnancy and socio-economic status (housing tenure, parental education and parental social class). Analyses were also further adjusted for fat and lean mass. To assess whether any of the risk factors for childhood fracture found in Chapter 13 ((vigorous physical activity, locomotor ability, time spent outdoors in summer, total energy intake, family size, birth weight or

maternal history of leg fracture) could be explained by an effect on bone size, these were added to the regression in a step-wise manner to see if any independent association remained. Participants with missing data were excluded from analysis. Stratified analyses were then carried out to look for evidence of effect modification or interaction (see Methods, Chapter 10, page 226). Interactions between variables were assessed by including a multiplicative interaction term in the regression models and calculating the likelihood ratio test (LRT).

14.3.7.3. Analysing the association between biomechanical strength indices and fractures

Logistic regression was used to calculate ORs and 95% CIs to describe the association between biomechanical strength indices and the risk of fractures over the following two years (see Methods, Chapter 10, page 225). Z scores were used to standardise an individuals biomechanical strength indices (see Methods, Chapter 10, page 221) so ORs are for fracture risk per SD unit change. The analyses were initially run adjusted for age at DXA measurement, and then progressively adjusted for gender, ethnicity, size of pregnancy and socio-economic status (housing tenure, parental education and parental social class). To assess whether any of the risk factors for childhood fracture found in Chapter 13 ((vigorous physical activity, locomotor ability, time spent outdoors in summer, total energy intake, family size, birth weight or maternal history of leg fracture) could be explained by an effect on biomechanical strength indices, these were added to the regression in a step-wise manner to see if any independent association remained. Participants with missing data were excluded from analysis. Stratified analyses were then carried out to look for evidence of effect modification or interaction (see Methods Chapter 10, page 226). Interactions between variables were assessed by including a multiplicative interaction term in the regression models and calculating the likelihood ratio test (LRT).

14.4. RESULTS

14.4.1. Basic description of the study population

Of the 5993 children in this Fracture Study, 2901 (48.9%) were male and 191 (3.5%) were non-white (see Results, Chapter 11, page 227). Comparison with the rest of the ALSPAC cohort shows preferential loss of male children and those of non-white ethnicity (see Results, Chapter 11, page 256).

The gender, ethnic and socio-economic distribution of the children in the Fracture Study have already been described in Results, Chapters 12 (page 267) and 13 (page 294). Table 56 on page 297 shows the association between gender or ethnicity and fractures and confirms boys and white children have a higher fracture risk. Table 64 on page 312 shows the association between socio-economic status and fracture risk.

14.4.2. Association between estimated volumetric bone density and fracture risk

There was a negative association between the two estimates of volumetric bone density and fracture risk (see Table 65, page 326). For the estimate of total body volumetric bone density adjusted for age at time of DXA scan, per SD decrease, fracture risk approximately doubled (OR 1.96, 95%CI 1.27 to 3.01, P=0.002). For the estimate of humeral volumetric density adjusted for age at time of DXA scan, per SD decrease, fracture risk increased by 26% (OR1.26, 95%CI 1.13 to 1.41, P<0.001). Neither of these estimates were changed after further adjustment for size of pregnancy, socio-economic status, gender or ethnicity. Analyses were also run after adjusting for fat mass (log transformed, see Results, Chapter 11, page 241) and lean mass with no change to the results.

In the analysis of the association between estimated volumetric density adjusted for age, gender, ethnicity, size of pregnancy and socio-economic status and fracture risk, gender and ethnicity remained independently associated with fracture risk suggesting that the observed associations are not mediated through an effect on estimated bone density.

Paternal education and paternal social class were no longer independent risk factors for fracture suggesting these act via an effect on estimated bone density.

To investigate whether any of the other risk factors for childhood fracture found in Chapter 13 (vigorous physical activity, locomotor ability, time spent outdoors in summer, total energy intake, family size, birth weight or maternal history of leg fracture) could be explained by an effect on estimated volumetric density, these were added to the regression of estimated total body volumetric density adjusted for size of pregnancy, socio-economic status, gender or ethnicity to see if any independent associations remained. Family size, vigorous physical activity, locomotor ability, total energy intake and birth weight were independent of estimated volumetric bone density. Maternal history of leg fracture and time spent outdoors in summer were no longer independent risk factors for childhood fracture, suggesting these act via an effect on estimated volumetric bone density.

Table 65: Association between estimated volumetric density measured at aged 9.8 years and fracture risk over the following two years

Measure of estimated volumetric bone density	Fracture Mean	SD	No fracture Mean	SD	Minimally adjusted OR OR 95%CI	P value	Adjusted OR (A) OR 95%CI	Adjusted OR (B) OR 95%CI	Adjusted OR (C) OR 95%CI	Adjusted OR (D) OR 95%CI
TBLH BMC adjusted for body size (g)	885	39	890	39	1.96 (1.27, 3.01)	P=0.002	1.96 (1.27, 3.01)	1.85 (1.11, 3.09)	1.92 (1.15, 3.21)	1.88 (1.12, 3.16)
Humeral vBMD (g/cm^3)	0.484	0.049	0.495	0.049	1.26 (1.13, 1.41)	P<0.001	1.26 (1.13, 1.410	1.28 (1.13, 1.46)	1.28 (1.12, 1.45)	1.27 (1.12, 1.45)

ORs are per SD decrease in the measure of estimated volumetric bone density. Minimally adjusted OR is adjusted for age at time of DXA measurement

(A) adjusted for size of pregnancy
(B) adjusted for socio-economic status and size of pregnancy
(C) adjusted for gender, socio-economic status and size of pregnancy
(D) adjusted for ethnicity, gender, socio-economic status and size of pregnancy

Abbreviations: BMC bone mineral content; CI confidence interval; cm centimetre; g gram; OR odds ratio; SD standard deviation; TBLH total body less head; vBMD volumetric bone density

14.4.3. Association between bone size and fracture risk

There was no association between any measure of bone size *per se* and fracture risk over the following two years (see table 66, page 329). However, there was a negative association between total body bone area and humerus length adjusted for body size (height and weight) and fracture risk. For the estimate of total body bone area adjusted for age at time of DXA scan, size of pregnancy, socio-economic status, gender, ethnicity and body size, per SD decrease, fracture risk increased by 62% (OR 1.62, 95%CI 1.23 to 2.15). For the estimate of humeral length adjusted for age at time of DXA scan, size of pregnancy, socio-economic status, gender, ethnicity and body size, per SD decrease, fracture risk increased by 25% (OR 1.25, 95%CI 1.01 to 1.54). Analyses were also run after adjusting for fat mass (log transformed, see Results, Chapter 11, page 241) and lean mass with no change to the results.

In the analysis of the association between bone size relative to body size adjusted for age, gender, ethnicity, size of pregnancy and socio-economic status and fracture risk, gender and ethnicity remained independently associated with fracture risk suggesting they do not mediated their effect via affecting bone size. Paternal education and paternal social class were no longer independent risk factors for fracture suggesting these act via affecting bone size.

To investigate whether any of the risk factors for childhood fracture found in Chapter 13 (vigorous physical activity, locomotor ability, time spent outdoors in summer, total energy intake, family size, birth weight or maternal history of leg fracture) could be explained by an effect on bone size relative to body size, these were added to the regression of estimated total body bone area relative to body size to see if any independent associations remained. Family size, vigorous physical activity, locomotor ability, total energy intake and birth weight were independent of bone size relative to body size. Maternal history of leg fracture and time spent outdoors in summer were no longer independent risk factors, suggesting these act via affecting bone size relative to body size.

14.4.4. Association between biomechanical strength indices and fracture risk

No measures of biomechanical strength of the humerus were associated with fracture risk over the following two years (see Table 67, page 330). Adjustment for size of pregnancy, socio-economic status, gender or ethnicity did not change this null association. Analyses were also run after adjusting for fat mass (log transformed, see Results, Chapter 11, page 241) and lean mass with no change to the results.

Table 66: Association between bone size measured at aged 9.8 years and fracture risk over the following two years

Measure of bone size	Fracture		No fracture		Minimally adjusted OR		Adjusted OR (A)	Adjusted OR (B)	Adjusted OR (C)	Adjusted OR (D)
	Mean	SD	Mean	SD	OR 95%CI	P value	OR 95%CI	OR 95%CI	OR 95%CI	OR 95%CI
TBLH bone area (cm^2)	1136	164	1136	164	1.02 (0.93, 1.12)	P=0.676	1.02 (0.93, 1.12)	1.05 (0.94, 1.17)	1.06 (0.95, 1.18)	1.62 (1.23, 2.15)
Humeral length (cm)	24.9	1.5	24.9	1.5	1.00 (0.90, 1.12)	P=0.967	1.00 (0.90, 1.12)	1.02 (0.90, 1.16)	1.00 (0.88, 1.14)	1.25 (1.01, 1.54)
Humeral width (cm)	1.92	0.17	1.90	0.17	0.93 (0.83, 1.03)	P=0.149	0.93 (0.83, 1.03)	0.92 (0.81, 1.05)	0.94 (0.82, 1.06)	0.97 (0.83, 1.14)
Humeral area (cm^2)	47.8	6.0	47.4	6.0	0.95 (0.85, 1.06)	P=0.342	0.95 (0.85, 1.06)	0.96 (0.84, 1.09)	0.96 (0.84, 1.09)	1.07 (0.87, 1.31)
Humeral AR	13.09	1.09	13.18	1.09	1.10 (0.98, 1.22)	P=0.096	1.10 (0.98, 1.22)	1.11 (0.97, 1.26)	1.08 (0.95, 1.23)	1.09 (0.95, 1.24)

ORs are per SD decrease in the measure of estimated volumetric bone density. Minimally adjusted OR is adjusted for age at time of DXA measurement

(A) adjusted for size of pregnancy
(B) adjusted for socio-economic status and size of pregnancy
(C) adjusted for ethnicity, gender, socio-economic status and size of pregnancy
(D) adjusted for body size (height and weight) ethnicity, gender, socio-economic status and size of pregnancy

Abbreviations: AR aspect ratio; CI confidence interval; cm centimetre; OR odds ratio; SD standard deviation; TBLH total body less head

Table 67: Association between biomechanical strength indices measured at aged 9.8 years and fracture risk over the following two years

Measure of biomechanical strength indices	Fracture Mean SD	No fracture Mean SD	Minimally adjusted OR OR 95%CI P value	Adjusted OR (A) OR 95%CI	Adjusted OR (B) OR 95%CI	Adjusted OR (C) OR 95%CI	Adjusted OR (D) OR 95%CI
CSMI	0.481 0.15	0.475 0.15	0.97 (0.87, 1.08) P=0.594	0.97 (0.87, 1.08)	0.97 (0.85, 1.11)	0.98 (0.86, 1.12)	1.05 (0.89, 1.25)
Relative bone strength	0.040 0.009	0.039 0.009	0.96 (0.79, 1.16) P=0.640	0.96 (0.79, 1.16)	0.95 (0.75, 1.20)	0.98 (0.77, 1.24)	1.06 (0.80, 1.40)

ORs are per SD decrease in the measure of estimated volumetric bone density. Minimally adjusted OR is adjusted for age at time of DXA measurement

(A) adjusted for size of pregnancy
(B) adjusted for socio-economic status and size of pregnancy
(C) adjusted for gender, socio-economic status and size of pregnancy
(D) adjusted for ethnicity, gender, socio-economic status and size of pregnancy

Abbreviations: CI confidence interval; cm centimetre; CSMI cross sectional moment of inertia; OR odds ratio; SD standard deviation

14.5. SUMMARY

These results provide the first prospective confirmation that bone mass is associated with fractures in children.

Estimated volumetric density from both total body DXA-data and regional data from the humerus show negative associations. The total body estimate has a larger increased risk of fracture per SD decrease compared with the humerus measure (96% versus 26%), perhaps explained by different measurement errors between the two techniques, and by smaller numbers of children having regional humerus measurements performed resulting in a less reliable estimate. Both these associations are consistent with the previous systematic review and meta-analysis of case-control studies (Literature Review, Chapter 7, Figure 19, page 164).

Bone size *per se* is not associated with fracture risk in children, but total body bone size relative to body size shows a negative association. The biological significance of this is unclear, but one possible explanation is that bone size relative to body size represents the level of adaptation of the skeleton to loading requirements i.e. the skeletal growth has not 'caught-up' with the growth of adipose tissue, muscle or height. No association was seen with humerus width, length or area, and this provides further data to the inconsistent literature on the association between bone size and fracture risk in children (Literature Review, Chapter 5, page 133).

Similarly, fracture risk was not associated with biomechanical indices of the humerus such as CSMI. One explanation may be that only a small percentage of childhood fractures occur in the humerus, and measures of skeletal geometry or biomechanical properties may relate to the risk of fracture at the site in question, while only providing limited information about fracture risk at other sites.

Family size, gender, ethnicity, vigorous physical activity, locomotor ability, total energy intake and birth weight were independent risk factors for fracture, independent of estimated volumetric bone density and bone size. Maternal history of leg fracture, paternal education and paternal social class were no longer independent risk factors, suggesting these act via affecting bone density or bone size. The initial positive

association between time spent outdoors in the summer and fracture risk, is no longer present after adjusting for bone mass, suggesting that more time spent outside in the summer reduces bone mass. This does not seem biologically plausible, and so it is likely that the association found between time spent outside in the summer and fracture risk is spurious, perhaps as a result of the multiple comparisons carried out in this thesis (see Discussion, Chapter 15, page 351).

14.5.1. Conclusions

This is the first report of a prospective cohort study of bone mass and fracture risk in childhood. It confirms the results of the previous systematic review and meta-analysis, and extends our knowledge by showing that both volumetric bone density and bone size relative to body size contribute to fracture risk in children. Family size, gender, ethnicity, vigorous physical activity, locomotor ability and birth weight were risk factors for fractures shown to be acting independently of volumetric bone density or bone size, so acting through another pathway such as by increasing the number of injuries the children experienced.

CHAPTER 15: DISCUSSION

The discussion chapter of this thesis reviews the original rationale and aims, summarises the main findings and discusses the main contributions of this research to the existing knowledge. This is followed by a discussion of the main strengths and weaknesses of this research, the implications of this research for paediatric bone health epidemiology, and the implications of this research for public health policy. Finally, directions for further research and the overall conclusions of this study are presented.

15.1. PROJECT RATIONALE AND AIMS

As described in Chapter 1, page 17, the rationale behind the Fracture Study was that childhood fractures are common and increasing, and can affect health and development. Although they are generally thought to reflect the fact that falls and other injuries are common in childhood, there is emerging evidence from case control studies that fractures in childhood are also related to underlying skeletal fragility. Research in this area is complicated by the difficulties estimating volumetric bone density and bone size from DXA measurements. Also, previous research into the effects of bone mass and other determinants has been mainly of a cross-sectional or case-control study design, and there is a need for detailed prospective data to examine the determinants of fracture risk in children and to explore the specific roles of bone density and bone size.

The aim of the Fracture Study was to carry out the first prospective investigation of the association between estimated volumetric bone density or bone size, and fracture risk over the following two years, in a large population-based birth cohort of children (ALSPAC). A preliminary stage to this was to investigate the confounding structure of childhood bone mass. A secondary aim was to investigate other determinants of fractures in children that may act independently of bone mass.

15.2. MAIN FINDINGS OF THIS RESEARCH

The clear social gradients in height, weight and fat mass observed, suggest that inequalities in both growth and adiposity are likely to be present in contemporary UK primary school children. Lower social position was associated with a greater body weight, a greater fat mass, and a shorter overall height in primary school children, but there was no social gradient in lean mass. At first sight, social position did not affect bone mass. However, after closer examination, the effects of social position on height and weight exert important, but opposing influences on bone size and bone mineral content in childhood. In other words, children born to mothers from lower social positions were shorter but heavier, while children born to mothers from higher social positions were taller but lighter: consequently the bone size and bone mineral content in children from low or high social positions were similar.

All measures of body composition were positively associated with measures of bone size. Height, lean mass and fat mass were positively associated with estimated total body volumetric density. Conversely, height and lean mass were negatively associated with humeral volumetric bone density while fat mass was positively associated i.e. taller children had a reduced estimated volumetric density at the humerus whereas fatter children had an increased bone density at this site, irrespective of pubertal status. These are the first investigations into the influence of fat mass and provide strong evidence that adipose tissue acts to stimulate both bone size by increasing periosteal growth, and volumetric density in children.

Estimated volumetric density from both total body DXA-data and regional data from the humerus show strong negative associations with fractures. Bone size *per se* was not associated with fracture risk in children, but total body bone size relative to body size showed a negative association. No association was seen with humerus width, length or area. Similarly, fracture risk was not associated with biomechanical indices of the humerus such as CSMI.

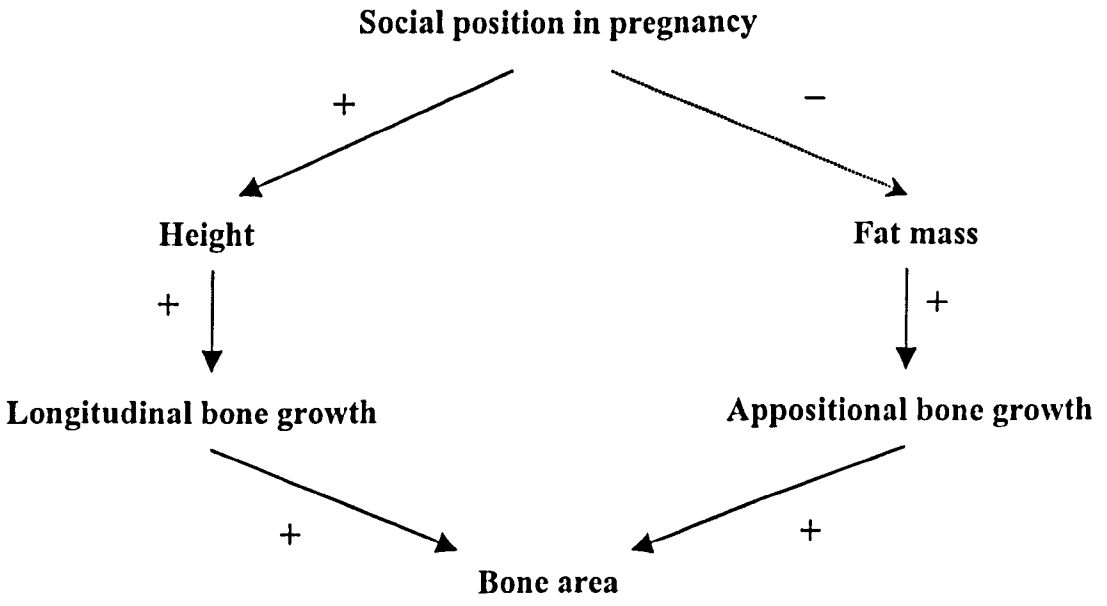
Family size, gender, ethnicity, vigorous physical activity, locomotor ability, total energy intake and birth weight were predictors of childhood fracture risk independent of estimated volumetric bone density and bone size. Maternal history of leg fracture, paternal education, paternal social class and birth weight were risk factors for childhood fracture risk that appeared to act via affecting childhood bone density or bone size.

15.3. CONTRIBUTION OF THIS RESEARCH TO EXISTING KNOWLEDGE

15.3.1. Social position and bone mass in children

This was the first study, as far as can be ascertained, to describe the association between social position and bone mass in children. Previous work in this area has been carried out in adult populations with contradictory results (302-308), perhaps because of a lack of inclusion of body composition data such as height and weight. The Fracture Study, using detailed body composition data, has increased our knowledge of the interplay between social position, body composition and bone mass. In other words, it is now known that whereas social position exerts a positive influence on BMC and bone area as a consequence of effects on longitudinal bone growth, this is opposed by a negative effect on appositional bone growth caused by associated changes in fat mass (see Figure 66, below).

Figure 66: Proposed relationship between social position in pregnancy and fat mass, skeletal growth and bone mass in offspring



Solid arrows represent positive associations and dashed arrow a negative association

15.3.2. Body composition and bone mass in children

The Fracture Study has shown that lean mass is a positive predictor of both total body BMC (r =0.898, see Results, Chapter 12, Table 54, page 279) and total body bone area (r =0.920, see Results, Chapter 12, Table 54, page 279), and this agrees with existing knowledge (see Literature Review, Chapter 3, page 89). This estimate for the coefficient between lean mass and TBLH BMC of 0.898 is lower than that of other studies such as 0.945 from a group of white children aged 5 to 18 years from the UK (314), or 0.98 from Australian children aged 4 to 26 (316), but similar to 0.88 from a study based on 9 year old Belgian girls (318). This may be explained by ALSPAC and the Belgian study consisting of children of the same age, perhaps suggesting that the association between lean mass and bone mass differs depending on age or pubertal stage. It is difficult to assess whether the size of the association between lean mass and TBLH bone area shown in this study are similar to those of existing studies, because of the use of CT by the other studies, rather than DXA to assess bone size.

However, the Fracture Study has shown that lean mass and height are negative predictors of estimated humeral volumetric density (see Results, Chapter 12, Figure 62, page 280). As already discussed (Results, Chapter 12, page 287) this is most likely to be due to an over-correction of BMC for size, rather than representing a true relationship. However, if the result found in the Fracture Study is true, this has not been previously reported, partly at least, because the humeral data used in the Fracture Study is derived from a novel method of sub-regional DXA analysis. However, the literature does suggest that the increased risk of fractures occurring around puberty may be due to a lag in bone mineralisation (i.e. density) relative to linear skeletal growth (15,508). Perhaps the negative association between lean mass or height and estimated volumetric density of the humerus shown in the Fracture Study, illustrates the temporal dissociation between development of long bone growth and bone mass during the growth spurt, which results in increased cortical porosity and a reduction in bone density (191). It is also possible that this temporal dissociation is most marked at the upper limb, perhaps explaining the preponderance of childhood fractures occurring in this skeletal area.

The results from the Fracture Study that fat mass is an independent positive predictor of both volumetric bone density (r = 0.665) and bone size (r = 0.685, see Results, Chapter 12, Table 54, page 279) helps to clarify this contentious area. The association shown in

the Fracture Study is less strong than those based on Italian children aged 5 to 17 years (r = 0.70) (317), and on 9 year old Belgian girls (r = 0.81) (318), but stronger than that based on Spanish girls aged 14 to 17 years (r = 0.38) (323), perhaps because of sample size or age considerations, as previously discussed.

In the results from the Fracture Study, a preferential action of fat mass at weight-bearing sites was not seen (see Results, Chapter 12, page 281), suggesting that mechanical load-bearing alone does not explain how adipose tissue might stimulate bone growth. This contradicts existing literature based on regional DXA analysis of girls aged 10 to 13 years from Finland (322), and CT-based analysis of 18 year old men from Sweden (211). In these two studies there was a stronger association between fat mass and bone mass in the legs compared to the arms, suggesting that mechanical loading is the major mechanism whereby fat mass affects bone mass. Potential explanations for the contradiction between these studies and results of the Fracture Study include the larger numbers in the Fracture Study compared to the other two studies (258 in the DXA study and 1068 in the CT-based study), providing a more precise estimate of the true association, or that the age differences and therefore pubertal stage differences of the study participants are important. It is possible that the association between fat mass and bone mass differs depending on pubertal stage.

Traditionally, gender differences in fat mass distribution are taken as representing sex hormone differences, as total fat mass and the proportion of trunk versus leg fat are greater in girls, presumably due to differences in levels of sex hormones (509). However, in the Fracture Study total body, trunk and trunk versus limb fat mass showed similar associations with bone size, and no interaction was observed with gender. Therefore, the Fracture Study provides no evidence that fat mass is acting as a surrogate marker for endocrine exposure in terms of its effects on bone size. Other mechanisms whereby adipose tissue might stimulate periosteal bone formation apart from mechanical loading or sex-hormones include leptin or other endocrine factors such as IGF-1 or oestrogen (fat mass in prepubertal children is related to serum levels of IGF-1 and oestrogen, both of which are known to influence skeletal growth (509)).

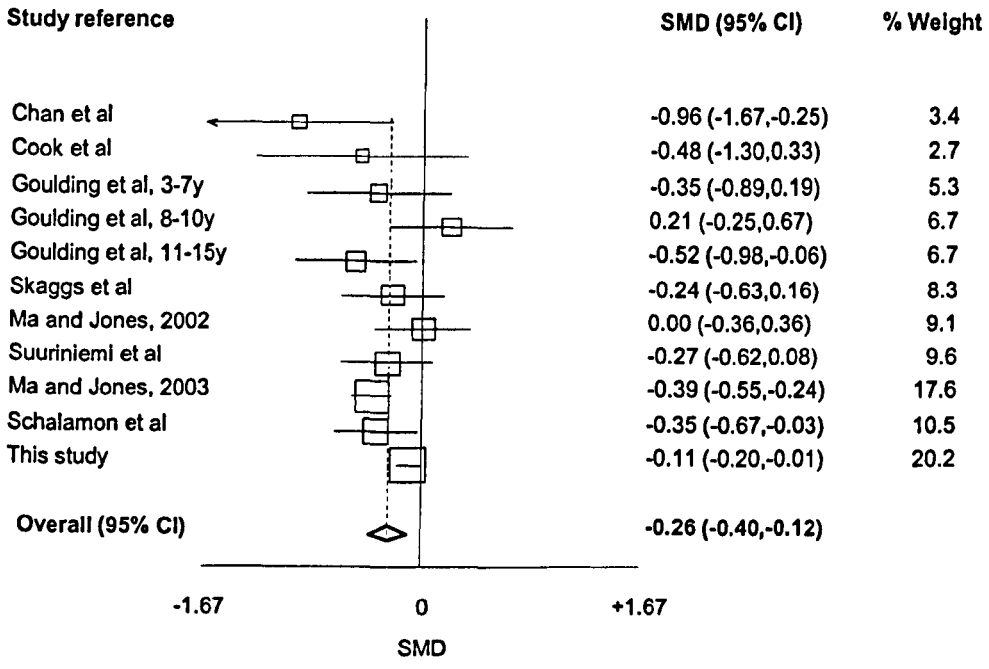
15.3.3. Bone density and fractures in children

The results from the Fracture Study provide the first prospective confirmation that bone mass is associated with fractures in children. Estimated volumetric density from both total body DXA-data and regional data from the humerus show negative associations. Bone size *per se* was not associated with fracture risk in children, but total body bone size relative to body size also shows a negative association with fracture risk over the following two years.

To examine how these results relate to those from previous studies, the meta-analysis (see Literature Review, Chapter 6, Figure 19, page 164) was repeated with the present findings included, based on results for TBLH BMC adjusted for body and skeletal size (see Figure 67, on the next page). In contrast to the original meta-analysis, there was evidence of heterogeneity (Chi-squared = 21.68 with 10 degrees of freedom, P=0.017), and so results were analysed using a random-effects model. The funnel plot showed no evidence of asymmetry. The SMD for the association between bone mass and fracture risk in children was marginally reduced from the original value of -0.32 to -0.26 (95% CI -0.40 to -0.12).

It is not clear why the effect of bone mass on fracture risk found in the Fracture Study was less strong than that observed in the majority of previous studies. The association reported in this study is likely to be more accurate due to the relatively large sample size and the prospective nature of the analysis, but the use of reported fractures rather than verified fractures may have reduced the strength of association found (see section on bias and misclassification, page 347). Another explanation is that the previous case control studies used different methods of measuring bone mass such as BMD by DXA or ultrasound.

Figure 67: Updated meta-analysis for the association between bone mass and fracture risk in children



The final model of TBLH BMC adjusted for age, gender, ethnicity, socio-economic status, height, weight and TBLH BA was used to generate the mean and SD for those children who did and did not report fractures (883g ± 38 and 887g ± 38, respectively).

Abbreviations: BA bone area; BMC bone mineral content; SD standard deviation; SMD standardised mean difference; TBLH total body less head

15.3.4. Risk factors for childhood fractures acting independently of bone mass

Gender, ethnicity, family size, vigorous physical activity, locomotor ability, total energy intake and birth weight were predictors of childhood fracture risk, independent of estimated volumetric bone density and bone size. They probably act by increasing the number of injuries the children experienced. The results observed in the Fracture Study for gender are consistent with the large body of literature that fractures are more common in boys compared to girls (see Literature Review, Chapter 5, page 125).

The association between ethnicity and childhood fracture risk is less clear (see Literature Review, Chapter 5, page 126), and the results from the Fracture Study add little to the literature, as they were based on only seven fractures in non-white children.

The results from the Fracture Study for the association between family size and childhood fracture risk increase our knowledge in this area, as only two previous contradictory studies have been found (456,459) (see Literature Review, Chapter 5, page 142).

The results from the Fracture Study of a positive association between vigorous physical activity and childhood fracture risk that is independent of bone mass, can perhaps help explain the contradictory literature (see Literature Review, Chapter 5, page 140). Data from RCTs show that physical activity in children increases volumetric bone density in the bones that are involved in the exercise (see Literature Review, Chapter 3, page 92), and this agrees with reports that light physical activity reduces fracture risk in children (466). However, the type of activity may be important. Physical activity with a small chance of injury probably increases bone mass and may reduce fracture risk (466), whereas vigorous physical activity or contact sports participation probably also increases bone mass, but because of the increased number of injuries also increases fracture risk (20,466). The previous literature is likely to be contradictory because the inter-related effects of physical activity, bone mass and injury exposure have not been fully explored.

The results from the Fracture Study for locomotor ability show that those children with the best locomotor ability at aged 4.5 years have an increased risk of fractures between aged 9.8 and 11.7 years. It is probable that the question on locomotor ability may identify those children with greater risk taking, who will try a new activity such as riding a bike without stabilisers (see Methods, Chapter 8, page 185) earlier than their peers, and so have an increased exposure to injuries. This agrees with the literature on the association between risk-taking behaviour and childhood fracture risk (see Literature Review, Chapter 5, page 137).

The positive association between total energy intake and fractures indicates that total energy intake may be a proxy for physical activity (as discussed in Results, Chapter 13, page 314), as those children who participate in more vigorous activity will be hungrier and eat more. However, the results from the Fracture Study do not agree with the single study in the literature that has investigated this area (463), and found an inverse association between total calorie intake and fractures in boys, but no association with

girls. An explanation for the differences seen is that the other study was based on only 127 children and was case control in design so is more likely to be biased than the Fracture Study.

The positive association between birth weight and fracture risk that is independent of bone mass has not been previously reported. At first sight it seems inconsistent with the literature on the positive association between birth weight and bone mass (see Literature Review, Chapter 3, page 79), but a potential explanation is that low birth weight babies are more protected by their parents and are more supervised during play, and consequently have a reduced number of injuries.

15.3.5. Summary

To summarise how the Fracture Study has contributed to existing knowledge about the determinants of fracture risk in children, the causal diagrams used to summarise the literature review (see Literature Review, Chapter 7, page 169) have been updated.

Determinants of fracture risk in children can be divided into three main pathways: bone fragility pathway, injury mechanism pathway and an exposure to injuries pathway. The Fracture Study has added knowledge to both the bone fragility and exposure to injuries pathways.

The updated bone fragility pathway is shown in Figure 68 on page 343. As in the previous causal diagram (see Literature Review, Chapter 7, Figure 21, page 172), the arrows represent the literature according to the epidemiological hierarchy of evidence (383). In addition, the knowledge added to this diagram by the Fracture Study is shown in red. The evidence for the association between socio-economic factors and bone density or bone size has gone from none, to evidence from cohort studies. Similarly, the association between fat mass and both bone density and bone size has gone from contradictory to that from cohort studies. For the association between lean mass and bone density the previous evidence was from case control studies, and for the association between lean mass and bone size was cross-sectional. Finally, the previous evidence for the negative association between bone density and fractures was from case control studies and this has been confirmed. The contradictory evidence for a negative

association between bone size and fractures has been clarified to be a negative association between bone size relative to body size and childhood fractures.

The updated exposure to injuries pathway is shown in Figure 69, page 344. Again, the knowledge added to this diagram by the Fracture Study is shown in red. There is no longer any strong evidence for the association between pubertal stage, obesity and psychological status or socio-economic status increasing exposure to injuries and increasing fracture risk in children, so these have been removed. Previous literature in these areas were based on cross-sectional analyses and was contradictory (see Literature Review, Chapter 7, Figure 23, page 176). The previous evidence on the association between family size and fracture risk was contradictory and this has now been clarified. The previous evidence for the association between physical activity and fractures was from case control studies and this has been confirmed. New evidence has been added showing that white ethnicity and higher birth weight increase exposure to injuries and so increase fracture risk.

Figure 68: Updated bone fragility pathway of fracture risk in childhood

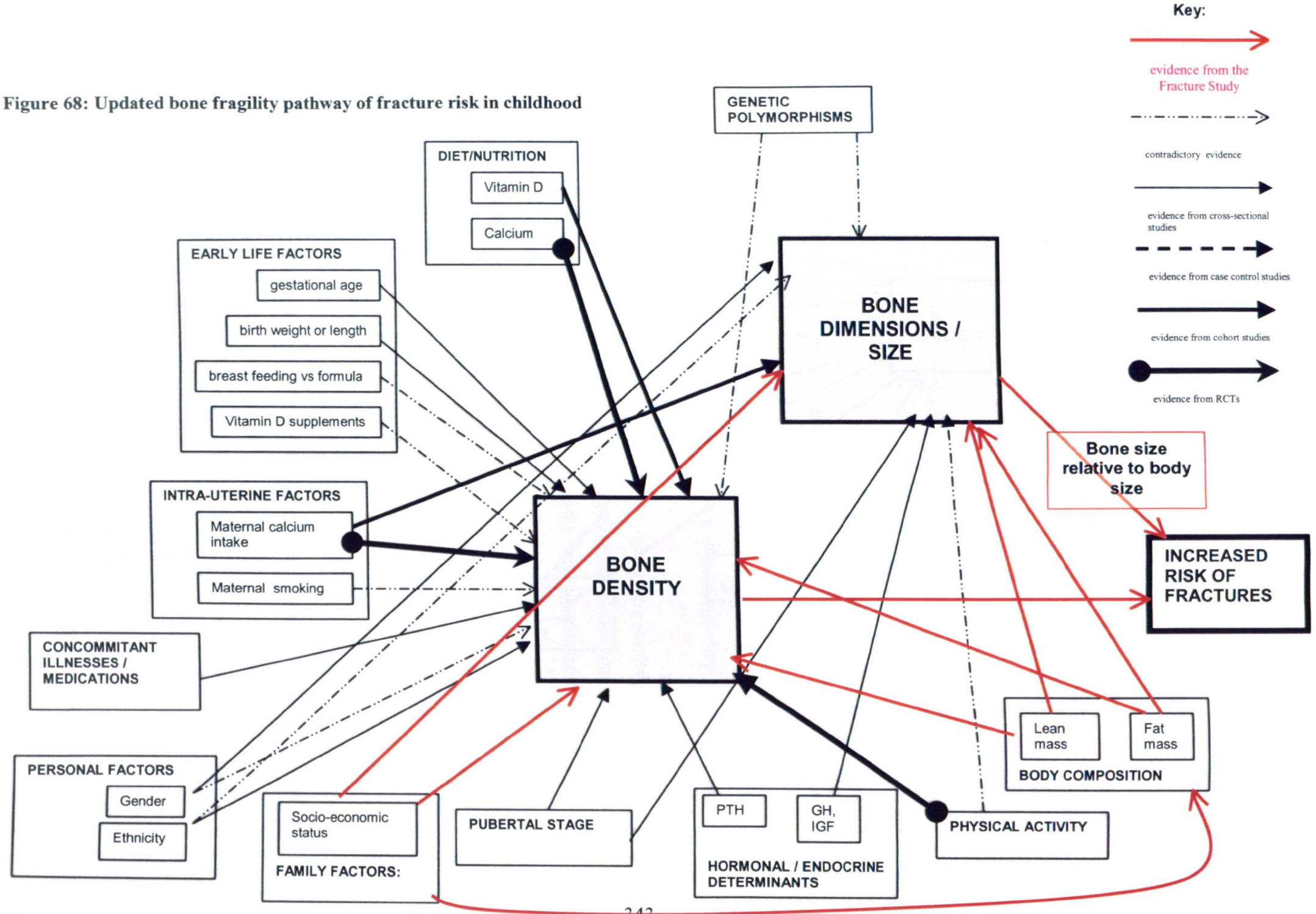
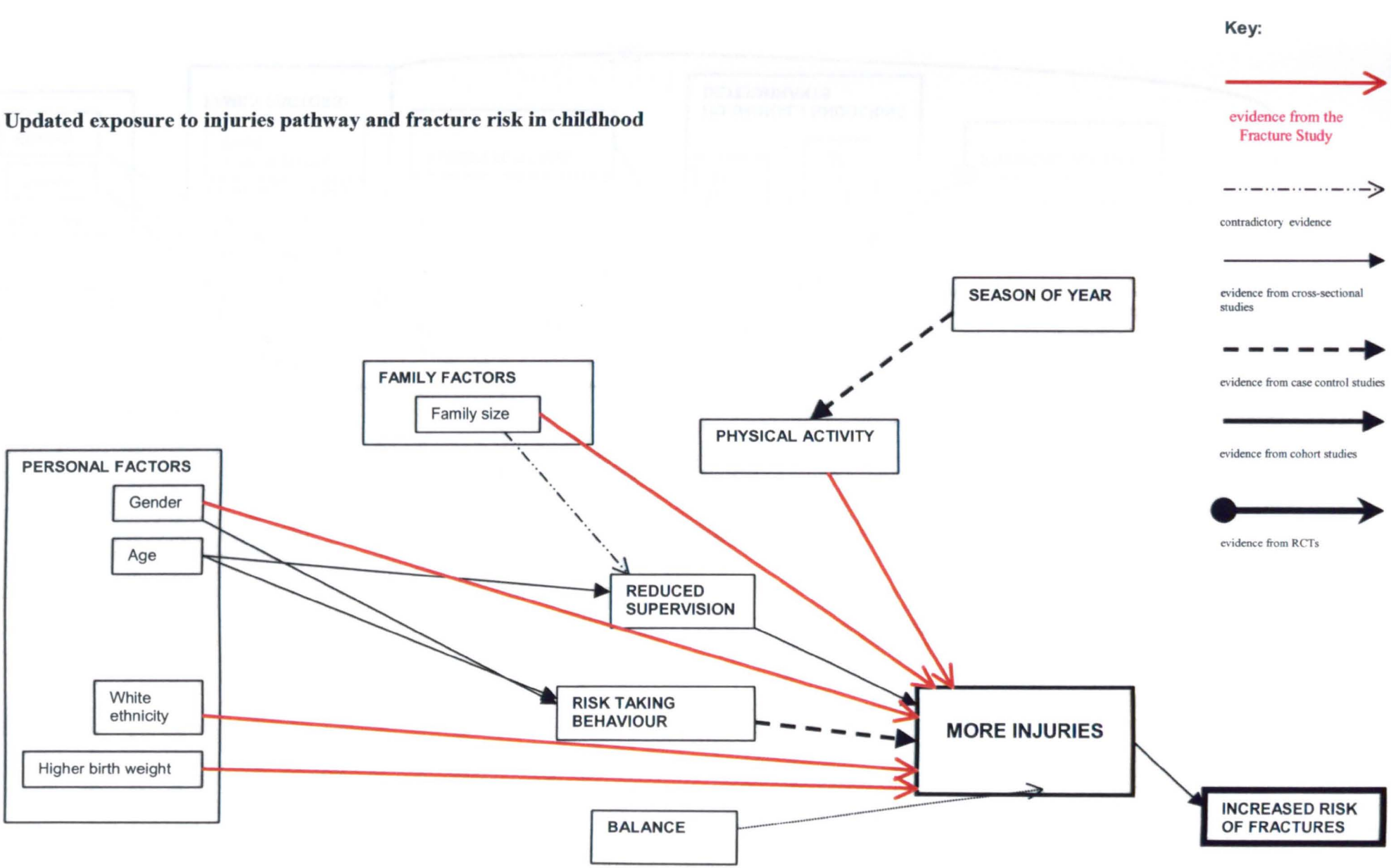


Figure 69: Updated exposure to injuries pathway and fracture risk in childhood



15.4. MAIN STRENGTHS OF THIS RESEARCH

15.4.1. ALSPAC

Data in ALSPAC were collected contemporaneously from questionnaires, medical records and from direct examination of the children in a research clinic setting, thus limiting any potential reporting or recall biases. Accurate equipment were also used to measure body size and composition, for example by DXA, and measuring equipment were calibrated daily, reducing the likelihood of systematic measurement error.

A particular strength of the Fracture Study was that the main analysis of the association between bone mass and fracture risk was prospective i.e. the data on estimated volumetric bone density and bone size were collected before data on reported fractures, ensuring exposure preceded outcome. In other words, unlike previous case control studies (see Literature Review, Chapter 6, page 147), in the Fracture Study the broken bone cannot have caused the lowered bone density through limitation of movement or change in subsequent behaviour.

The wealth of data collected in ALSPAC also permitted a comprehensive approach to investigating the determinants of fractures in children, including the assessment of the effect of confounding, and detailed work on potential causal pathways. Also, results from ALSPAC have a contemporary relevance and should not be affected by secular trends in anthropometry and other factors.

15.4.2. Sample size and generalisability to all UK children

The number of children with data on fractures included in the Fracture Study was large (n=5933). In addition these children were healthy. The original ALSPAC population was fairly representative of that of the UK (see Methods, Chapter 8, page 182), but the Fracture Study is based on only 42% of the original ALSPAC cohort alive at 12 months (see Methods, Chapter 8, Figure 24, page 181). This large drop-out is likely to have resulted in selection bias which does not affect the internal validity of this study, but

does affect the generalisability of the results found (see section on selection bias on page 349).

This study may not be generalisable to the whole of the UK, or to low- or middle-income countries because of the differences between children who took part in this study and children who did not, and because of missing data. These missing data did not occur through random misfortune such as losing forms, but through a family's/child's decision not to answer a questionnaire. This means that the families/children who did not answer a particular questionnaire are likely to be an atypical subgroup. However, it is possible to define the group of children from ALSPAC who answered questions on early life exposure variables, for example, and from this define the population of children who reported whether or not they had a fracture during aged 9.8 to 12 years. Therefore, missing data does not affect internal validity of this study, but does affect the generalisability of the results found. However, if the associations found in this study are causal, they are likely to be working through biological mechanisms which will be similar in all humans, irrespective of socio-economic, ethnic or other background. It is possible that missingness could introduce bias if the association between exposure and outcome is different in those that took part compared to those who didn't, but this is considered unlikely.

15.4.3. Statistical strengths

In addition to the large sample size, the statistical strength of the Fracture Study were that all analyses were conducted using appropriate statistical modelling, and associations with fractures were explored before and after adjustment for potential confounding factors.

15.5. POTENTIAL LIMITATIONS OF THIS RESEARCH

15.5.1. Confounding

In common with all cohort studies, a major threat to the validity of the findings of the Fracture Study is confounding, both measured and unmeasured. Confounding is a distortion of the estimated effect of an exposure on an outcome caused by the presence of an extraneous factor associated with both the exposure and the outcome (383). A discussion of the effect of using poorly measured known confounders in the analyses is presented in the next section below.

The most likely candidates for potential unmeasured confounders in the association found between body composition and bone mass, include hormonal factors such as leptin, or perhaps functional genetic polymorphisms that affect fat mass, lean mass and bone mass. For example, the association between fat mass and bone mass may reflect an indirect influence of other factors responsible for increased energy intake or decreased energy expenditure that underlie obesity.

The most likely candidates for potential unmeasured confounders in the association between factors other than bone mass and fracture risk include injury exposure such as the number of falls each child experienced, or perhaps clumsiness or awkwardness during falling. For example, vigorous physical activity is likely to increase the number of injuries a child experiences, and therefore increase fracture risk, but the children who fracture may be the clumsy ones who fall awkwardly.

15.5.2. Bias and misclassification

Bias or misclassification can also affect the validity of these results. Bias is a deviation of the results from the truth (383).

15.5.2.1. Information bias and misclassification

Information bias is a flaw in measuring exposure or outcome data (383). Bias in the measurement of the main exposure variable of bone mass or body composition is

unlikely to have occurred, as the DXA scans were carried out carefully with appropriate quality assurance and staff training (see Methods, Chapter 8, page 189). Repeatability tests showed results marginally better than the machine manufacturers would expect. 102 scans of the 7444 (1.4%) performed were unusable which may produce bias if these children had different distributions of bone mass than the population used in the Fracture Study, but is unlikely.

Bias or misclassification of the outcome, fractures, is likely as *reported* fracture was used, not fully verified fracture by X-ray examination. Examining X-ray reports (Methods, Chapter 9, page 216) showed that approximately 82% of these reported fractures were likely to be true fractures diagnosed on X-ray and 18% misclassified as having fractures. No information is available on the number of children who denied a fracture, but actually had one diagnosed on X-ray. This misclassification of children into those who fractured and those who didn't is likely to be random, and as such is likely to reduce the magnitude of any association found, rather than produce a spurious result i.e. the estimate of an 89% increased risk of fracture per SD decrease in estimated volumetric bone density is likely to be an under-estimation of the true association.

Bias in the answering, collection or recording of answers to the questions about the other variables or confounders used, may also threaten the validity of this study. Data on gender, ethnicity, gestational age, size of pregnancy and birth weight is unlikely to be biased as it was collected from medical notes and birth notifications as well as via questionnaires sent to the mother. The question on maternal smoking during pregnancy is asking about a socially undesirable behaviour, and some mothers may not have answered truthfully. This may therefore invalidate the result of no association between maternal smoking during pregnancy and fractures found in this study.

Bias in the answering, collection or recording of answers to the questions about socio-economic status may have occurred as the questions on socio-economic status may have been perceived as intrusive, and some mothers may not have answered truthfully resulting in erroneous misclassification of some individuals. However, any misclassification of socio-economic status due to this is likely to be random, i.e. not associated with the outcome of body composition, bone mass or fractures. Random

misclassification reduces the strength of associations found, but does not produce spurious results.

There is evidence that pubertal status, particularly scrotum/genital development for boys is biased (see Results Chapter 11, page 237), and this unreliability of the pubertal measure may explain why no association was found between pubertal status and fracture risk.

The question on vigorous physical activity per week may also have bias. Parents who filled in the questionnaire may not have intimate knowledge of exactly what exercise their child does per week. Also, the question on vigorous physical activity (see Methods, Chapter 11, page 232) covers a wide range of pursuits including swimming. Swimming is non-weight bearing and has been shown to be associated with lower bone mass than other types of activity (510). This may have resulted in non-differential or random misclassification and again reduce the strength of associations found.

The data on paternal factors may also have been biased, as all paternal data were collected from questionnaires to the mothers. Again, this may result in random misclassification of paternal weight, paternal height and a history of paternal fractures, and may explain the null associations shown in the Fracture Study.

15.5.2.2. Selection bias

Selection bias is an error due to systematic differences in characteristics between those who take part in a study and those who do not (383). As discussed under generalisability (page 345), the Fracture Study is based on only 42% of the original ALSPAC cohort alive at 12 months (see Methods, Chapter 8, Figure 24, page 181). This large drop-out over 12 years did not occur randomly, and there was preferential loss to those children whose parents were from lower socio-economic backgrounds (see Results, Chapter 11, page 256). Also, these children who agreed to have, and attended for a DXA scan were likely to be different to the children who did not attend for a DXA scan i.e. a non-random sample was used for this Fracture Study. Selection bias due to the large drop out may invalidate any conclusions about the association between social position and body composition or bone mass found in the Fracture Study. However, although there was a disproportionate loss of those children born to mothers of lower

social position, this would only have led to a spurious association between social position and bone development if the relationship between social position and bone growth was different among those children who were lost compared with the remainder of the cohort, which is considered unlikely. Also, this selection of study participants might be expected to reduce social gradients, but the results from the Fracture Study show clear gradients exist for height and weight.

15.5.2.3. Recall bias

All data on risk factors were collected by contemporaneous questionnaires, and collected prior to data on reported fractures was collected, so knowledge of whether the child has fractured or not has not influenced data collection on risk factors. This has ensured the correct temporal sequence between the potential risk factors and fractures, and will have reduced the likelihood of any recall bias serious enough to affect the outcome of this study. However, some potential risk factors may change over time (see next section below) and this may make interpretation of results difficult.

15.5.3. Time lapse between exposure variables and outcome

Timing of the questions during childhood can make interpretation of the results difficult. For example, whether the associations found between social position, body composition and bone mass reflect an action of social position *in-utero* or in early childhood i.e. programming, is uncertain, as the precise relationship between social position in pregnancy and subsequent childhood is unclear.

Some of the variables, such as the data on risk avoidance, may have changed between the time the question was asked, and aged 9.8 to 11.7 years when fractures were recorded. For example, this question on risk avoidance was asked when the children were aged 3.5 years. Whether the answer to this question accurately reflects the child's personality or risk taking behaviour when aged 9.8 to 11.7 years is debateable. This may explain why no association was found between risk avoidance and fractures.

Some of the variables may not reflect exactly what is expected i.e. may not be valid and therefore affect interpretation of any association found. For example, total energy intake is most likely to be associated with fractures because energy intake reflects increased

physical activity and therefore an increased number of injuries rather than a direct effect of energy intake on fractures. The data on breast feeding is not *exclusive* breast feeding (i.e. no supplementation with baby milk formulae, and no weaning) and this may explain the null result found for its association with fracture risk.

15.5.4. Random error or chance

An alternative explanation for the associations found is random error or chance, as this is always possible in observational studies. However, in the Fracture Study there were established *a-priori* hypotheses. The sample size was large and 95% confidence intervals were small. Despite this, the multiple comparisons and associations investigated during the Fracture Study make it more likely that some of the results found could have been spurious and occurred by chance, such as the association found between time spent outdoors in summer and fractures (see Results, Chapter 14, page 332).

15.5.5. Use of odds ratios

A further concern is that with a fracture rate of 8.9%, this is getting to be a 'common' outcome, so odds ratios may tend to over-estimate underlying associations. However, in general, in medical literature the statistical analysis of binary outcomes (such as fracture or no fracture) is almost always based on odds ratios, even when the outcome is common. In this situation, although odds ratios may tend to over-estimate the size of effect (492), computational problems and difficulties with interpretation do not occur, as would otherwise be the case if risk ratios were used instead. An additional benefit of using odds ratios is that the conclusions will be identical whether we consider the outcome as the occurrence of an event, or the absence of an event.

15.5.6. Errors in the Fracture Study design and protocol

The humerus sub-regional analysis to produce measures of humeral bone size and estimates of humeral volumetric density and biomechanical strength was based on only a sample of the Fracture Study children, and this is a shortcoming. Reduced numbers may explain the null associations found between the measures of biomechanical

strength and fracture risk, although estimated volumetric density of the humerus had a clear negative association with fracture risk.

Use of total body DXA scans to assess BMC and bone area in the Fracture Study meant that only estimated volumetric bone density could be used, as opposed to directly measured volumetric bone density, and this is a compromise. However, to directly measure volumetric bone density in children, a pQCT scan would be needed, and although this gives a similar dose of radiation as a total body DXA scan (see Literature Review, Chapter 2, table 1, page 47) it has disadvantages, particularly expense, and can only be used on one bone of the skeleton e.g. tibia.

The use of reported fractures rather than verified fractures will have reduced the strength of any associations found. An alternative protocol would have been to obtain multi-centre REC approval so that copies of all X-ray reports could have been obtained, irrespective of which hospital in the country the children were X-rayed. This would have been time-consuming, but would have allowed fully verified fractures to have been used as the outcome and increase accuracy of the results.

15.5.7. Statistical weaknesses

The main statistical weakness of the Fracture Study was that large numbers of comparisons were performed, and so some of the associations observed may have been spurious. This is likely to be the case with time spent outdoors in summer and fracture risk. It may have been better to define a conservative statistical significance threshold of less than 0.1% i.e. $P<0.001$. Had this been the case the associations between gender, ethnicity, birth weight, maternal history of leg fractures and family size, with fractures would no longer have been significant. However, the main prospective analyses would not have changed i.e. there would still have been a significant association observed between social position and body composition, social position and bone mass, body composition and bone mass, estimated volumetric bone density and fractures, bone size and fractures and vigorous physical activity and fractures.

Another statistical weakness is that no imputational methods were used to account for missing data (see Methods, Chapter 10, page 224 on missing data approaches). Instead, participants with missing data were excluded from analysis. Approaching missing data

with this method will reduce power due to loss of numbers, but is unlikely to result in spurious associations.

15.6. IMPLICATIONS OF THIS RESEARCH

15.6.1. For paediatric bone health epidemiology

One implication of the Fracture Study for paediatric bone health epidemiology is that reported fracture can be used as a proxy for verified fracture when carrying out studies of children.

Another implication, not only for paediatric bone health epidemiology but also for adult epidemiology is that detailed regional sub-analysis of the humerus on total body DXA scans using the Region of Interest tool is possible, practical and accurate. The Fracture Study shows that this method can provide epidemiologists with further tools to investigate bone dimensions without resorting to more expensive investigations such as CT. The Fracture Study also shows that it is possible to distinguish between estimated bone density and bone size using total body DXA data.

This research also highlights the importance of collecting additional data such as socio-economic status or body composition, including fat mass, for epidemiological studies investigating both volumetric bone density and bone size in children, and of exploring the effect of adjusting for these.

15.6.2. For adult bone health epidemiology

Some of the results from the Fracture Study may be extended to adults. For example, perhaps bone size relative to body size is a novel risk factor for adult fractures. There is also the possibility that childhood fractures themselves are a risk factor for adult fractures, including osteoporotic fractures.

15.6.3. For public health policy

The implications of the Fracture Study for public health policy are interesting, and can be used as a reminder that discipline-specific advice such as from a paediatric bone health epidemiological study, should not be used in isolation to formulate public health policy. Instead, a range of views should be taken into account.

The results from the Fracture Study suggest that increasing volumetric bone density or bone size relative to body size may help to reduce fracture incidence in children. Furthermore, this study observed that both lean mass and fat mass are independent positive predictors of volumetric bone density and bone size in childhood. This raises the concern that current proposed interventions intended to reduce childhood obesity and therefore reduce the impact of type II diabetes mellitus and ischaemic heart disease in later life (511), may have adverse consequences for skeletal development and the risk of osteoporotic fracture in later life. However, suggesting that children should increase their adiposity to aid their bone health is clearly inappropriate. Understanding the mechanisms involved in this apparent link between fat mass and periosteal growth may suggest strategies for limiting any such adverse impact.

The results from the Fracture Study also suggest that reducing the number of injuries a child is exposed to, will help to reduce fracture incidence independently of bone mass. However, limiting the amount of vigorous physical activity a child carries out per week, which is the proxy for exposure to injuries used in the Fracture Study, is also inappropriate. Perhaps increased supervision by adults and awareness of the potential increased fracture risk for children during sports could help reduce the incidence.

15.6.4. For individuals

The results from epidemiological studies such as the Fracture Study can be used to guide decisions about individuals, but they relate primarily to populations, and as such extrapolation must be made with care (512). The main aim of cohort studies is to show in which areas intervention studies should be focussed. Interventional studies such as RCTs provide good information for individuals. However, the results of the Fracture Study can give clues to what are likely to be risk factors for fractures in individuals, such as volumetric bone density, but do not attach clinical importance to the risk factor.

For example, taking all the previous literature and the results of the Fracture Study it seems reasonable to conclude that volumetric bone density is negatively associated with fractures in children. However, no evidence is available as to how many less children would fracture bones if they increased their bone density by 10% for example. For this further research is required.

15.6.5. For future research directions

Initially, further observational studies are required to expand our knowledge on the determinants of bone mass in children. These studies should be carried out on large populations of children, and should accurately measure hormonal levels such as leptin and IGF-1, and measure functional genetic polymorphisms, as well as other variables such as gender, ethnicity, size of pregnancy and puberty measured in this study.

Further observational studies are also required to expand our knowledge on the risk factors for fractures in children. These studies should also be carried out on large populations of children, and should accurately measure the total number of injuries that occur, irrespective of outcome, as well as other variables such as gender, physical activity and family size measured in this study. The large populations should be chosen to contain a reasonable proportion of children with diverse ethnic backgrounds to allow further exploration of the role of ethnicity. Other observational studies should also be carried out to try and assess more accurately the role of risk taking behaviour and puberty.

Further careful observational studies are also justified to examine the complex inter-relationships between body composition, bone size and fracture risk in children. The association between fat mass and height-adjusted bone area, which has been interpreted as reflecting an association between fat mass and periosteal bone growth, requires confirmation by further studies in which cross sectional area is measured directly using techniques such as pQCT, although some validation of this assumption is provided by the positive association between fat mass and width of the humerus.

The overall aim of these further observational studies should be to accurately guide the set-up of randomised, blinded, controlled interventional studies of specific activities in

children aimed at increasing bone mass. Examples may include modulation of body composition by exercise or diet. Then, further intervention studies should focus on specific activities in children aimed at reducing exposure to injuries, with fractures as the outcome. Examples may include increased supervision during play, perhaps targeted to those children with high risk-taking behaviour.

Finally, further studies should include randomised, blinded, controlled interventional studies of specific activities in children aimed at increasing bone mass or bone size, with fractures as the outcome. Examples of potential determinants of bone mass from this thesis include lean mass, perhaps increased by physical activity. There have been some previous RCTs of school-based exercise programmes (342,344,513) which do increase childhood bone mass, but follow-up has been limited, and fractures have not been used as an outcome.

The findings of the Fracture Study could be assessed in different populations, for example in adults. Further studies into whether bone size relative to body size is an independent risk factor for adult fractures would be important.

15.7. CONCLUSIONS

In conclusion, bone fragility as measured by volumetric bone density and bone size relative to body size, is a determinant of fracture risk in children. However, other risk factors, acting via increased exposure to injuries or via the mechanism of injury, are also important. Public health interventions aimed at reducing the incidence of childhood fractures needs to encompass all three areas. Future studies in this area must be aware of the complex associations between body composition, bone fragility and fracture risk in children.

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